

FREQUENCY-DEPENDENT HOST CHOICE BY  
PHYTOPHAGOUS INSECTS AND THE EVOLUTION  
OF PLANT DEFENCE STRATEGIES

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Adrian Monks

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## ABSTRACT

Herbivore adaptation to plant resistance presents an evolutionary trade-off for plants. In the absence of plant resistance the plant is susceptible to non-adapted herbivores. However herbivores that are adapted to a particular plant resistance trait may increase the cost to the plant of expressing the trait if the herbivores use it as an attractant. The adapted herbivore escape hypothesis suggests that plants may get around this problem by only deploying the resistance when necessary in response to herbivore feeding. This hypothesis requires that adapted herbivore are able to discriminate in favour of resistant plants when making host choice decisions and that host selection is frequency-dependent such that the undamaged phenotype can escape when rare.

This thesis set out to determine how host phenotype frequency affects choice by insect herbivores in both a mechanistic and a functional sense. In addition, a study was made of the conditions under which induced strategies may be evolutionary stable when under selection by adapted and non-adapted herbivores in the absence of physiological costs of defence.

The role of learning and host-deprivation in the oviposition acceptance behaviour of the diamondback moth (*Plutella xylostella* L. (Lepidoptera: Yponomeutridae)) was investigated. Previous experience with a host plant increased acceptance in a host-specific way. Discrimination between host plants was independent of deprivation. Existing models of insect oviposition acceptance do not adequately explain these findings. A new model – The Incremental Acceptance Model – is proposed.

In laboratory trials containing mixtures of damaged and undamaged *Brassica oleraceae* L. and *B. napus* L. plants at different frequencies, a key assumption of the adapted herbivore escape hypothesis was supported. The probability of oviposition by the diamondback moth was dependent on the frequency-of the damaged state and undamaged *Brassica* plants were more likely to escape herbivory than were damaged plants. The larval rearing plant affected the oviposition preference of the moths for damaged plants.

The evolution of plant defence strategies was modelled under frequency-dependent selection by non-adapted and adapted, or partially adapted herbivores. A stable state always arose that contained both the defended and undefended plant phenotypes, except under certain restrictive conditions. Inducibility formed a pure ESS provided the defended phenotype was not fitter than the undefended phenotype and the plant defence reduced the feeding damage by non-adapted herbivores. In contrast with previous models, induction is not necessarily favoured when herbivory is predictable or the costs of expressing defence intermediate. It is argued that frequency-dependent herbivory is a major omission from previous studies of the evolution of plant defence strategies.

## CHAPTER 1

### INTRODUCTION

#### ***BACKGROUND***

Herbivore inflicted injury represents a significant cost to plants. It has been estimated that the median level of the annual primary productivity of terrestrial plants that is removed by herbivores is 18% (Cyr & Pace 1993; mean approx. 34% calculated from Cyr and Pace's data). This biomass removal translates into negative effects for numerous indicators of plant performance (Crawley 1983, 1985; Islam & Crawley 1983; Marquis 1992; Maurico *et al.* 1993; Root 1996; Wise & Sacchi 1996; Mauricio *et al.* 1997) and can affect the composition of plant communities when particular plant species are targeted (*e.g.* Parker & Root 1981; Bowers 1993). Not surprisingly, herbivores can select for resistance traits that reduce damage to the plant by decreasing herbivore preference or performance (Mauricio & Rausher 1997). Two broad classes of resistance are recognised. The first is constitutive resistance, in which the resistance trait is constant relative to the damage inflicted by plant enemies. This definition does not imply that these resistance traits are always constant however, as they may change over developmental and evolutionary time. By contrast, inducible resistance is expressed only in response to damage to plant tissue by plant enemies. The magnitude and the qualitative components of the induced response are determined by the nature of the damage (Karban & Baldwin 1997).

#### ***THE EVOLUTION OF INDUCED RESISTANCE***

The constitutive/induced dichotomy in the expression of plant resistance characteristics has produced considerable and ongoing debate as to the functional merits of the two strategies (Fowler & Lawton 1985; Karban & Myers 1989; Herms & Mattson 1992; Karban & Baldwin 1997). It is widely assumed that constitutive resistance traits are more effective at reducing herbivory by susceptible herbivores because there is no time lag prior to activation as occurs with induced resistance (Karbon & Baldwin 1997; Baldwin 1998). Accordingly, to explain the evolution of induction, many hypotheses have been proposed to counter this perceived trade-off against effectiveness.



Dominant in the literature has been the argument that compared with resistance expressed constitutively, inducibility reduces the physiological costs associated with being defended because there is a cost of resistance only when the plant requires defending (Karban & Myers 1989; Karban & Baldwin 1997; Karban *et al.* 1997). A considerable body of work has focused on estimating the resource costs of defensive compounds and structures (Gershenzon 1994), and there may be significant opportunity costs if these resources are limiting (Gulmon & Mooney 1986). In addition, there may also be costs associated with auto-toxicity (Till-Bottraud & Grouyon 1992), both in terms of damage to plant cells, and the need for structures to contain the toxins. Generally however, these costs do not seem to translate into widespread negative consequences for plant fitness. A significant growth and fitness cost of defence has been reported in some instances (Mauricio & Rausher 1997; Mauricio *et al.* 1997; Baldwin 1998; Mauricio 1998), but in others a cost of resistance has been detected in only some components of plant fitness (Agren & Schemske 1993; Agrawal *et al.* 1999) or in none at all (Simms & Rausher 1989; Karban 1993; Gianoli & Niemeyer 1997). Of the 50 studies containing 88 comparisons reviewed by Bergelson and Purrington (1996), evidence for a physiological cost to resistance was found in only 56% of cases for resistance to microbes and 29% of resistance to insects. The cost hypothesis also predicts a negative correlation between tolerance to herbivores and allocation to resistance if resources are limiting. Agreement with this prediction is also equivocal - such a correlation has been found by some studies (*e.g.* Fineblum & Rausher 1995; Stowe 1998) but not others (*e.g.* Mauricio *et al.* 1997; Agrawal *et al.* 1999; reviewed Strauss & Agrawal 1999). The failure of the cost hypothesis to generally account for induction may be in part due to the lower than expected cost for the ongoing maintenance of chemical defences because of metabolite recycling and unexpectedly low rates of metabolite turnover (Gershenzon 1994). It has also been suggested that plants may have subsequently evolved physiological mechanisms to minimise these costs and hence the original factors driving the evolution of induction may no longer be observable (Simms 1992). While this could conceivably be so, it also has the unappealing side effect of rendering the cost hypothesis untestable. On the current evidence, the physiological cost of defence is probably not a significant factor in the evolution of induction for many plants (Karban & Baldwin 1997).

Many alternatives to the cost hypothesis have been proposed. The supply and demand hypotheses - the carbon-nutrient balance hypothesis (Bryant *et al.* 1983; Coley *et al.* 1985) and the more general growth differentiation balance hypothesis (Loomis 1953; Tuomi *et al.* 1990; Herms & Mattson 1992) - are mechanistic variants on the cost theme. They are

included separately from the cost hypothesis as they do not presume that the resource cost is the selective factor for the evolution of induction. Rather, these hypotheses propose that induction is a physiological side-effect arising from the herbivore generated imbalance between supply and the priority demands of growth for carbon and nutrients. It is postulated that these plant responses may have originally evolved as mechanisms for coping with stress and that they have secondarily become implicated as responses to herbivory. In support of these hypotheses, fertilisation prior to defoliation in nutrient limited systems can in some instances mitigate an induced response to herbivory (Wilcox & Crawley 1988; Bryant *et al.* 1993) and there is some circumstantial evidence that shade decreases the concentration of carbon based compounds (*e.g.* Bryant *et al.* 1987; Nichols-Orians 1991). By contrast, other studies have found no evidence that the induced response is affected by fertilisation prior to defoliation (Haukioja & Neuvonen 1985; Neuvonen & Haukioja 1991) or the removal of the carbon source (Honkanen *et al.* 1999). In addition, nitrogen limited plants have also been shown to increase their allocation to nitrogen based compounds relative to the rate of carbon fixation following induction (Baldwin & Ohnmeiss 1994). Those studies which found no trade-off between defence and growth also argue against the mutually exclusive dichotomy predicted by these hypotheses (Mauricio *et al.* 1997; Agrawal *et al.* 1999).

In the face of the very equivocal evidence for the resource-based hypotheses, a growing number of hypotheses do not assume a physiological cost to defence. In one proposal it is argued that constitutive resistance is simply a specialisation of induction. According to Karban and Myers (1989), induction may have been the ancestral condition and constitutive resistance is just an induced response without an off switch. They argue that the constant presence of pathogens on the surface of the plant selects for an invariable defensive response as the plant is continuously challenged. This theory has received little attention, possibly because it is largely untestable.

Recently Karban *et al.* (1997) proposed that variable defence investment may be more effective at reducing herbivore load than a single fixed level of defence. Karban *et al.* contended that provided maximal levels of constitutive defences are sufficiently constrained, and the 'benefit' to the herbivore is a negative and convex function of the concentration of toxin, then the average net 'benefit' to the herbivore population feeding on plants which have two or more defence levels is lower than the net benefit from a single level of toxin. This hypothesis cannot explain the presence of induction on its own because it requires the level of constitutive defence to be constrained, but it does suggest that variation in defensive states

between individuals provides an extra benefit to inducible populations. This hypothesis does not appear to have been tested yet.

In the natural enemies hypothesis, Dicke and Sabelis (1989, cited in Karban & Baldwin 1997) suggested that inducible plants may have lower herbivore loads when the natural enemies of the herbivore use the response as an attractant. By only inducing when being eaten, the search efficiency of these natural enemies will be improved and the parasitism/predation rate of the herbivore increased. There is considerable evidence that herbivore enemies are both attracted to induced plants, and that the herbivores suffer a corresponding increase in the incidence of parasitism (Turlings *et al.* 1995; Dicke 1999; Thaler 1999). However, it is difficult to see how this could select for induced resistance when constitutively defended individuals would also benefit from such a scheme. Induction could not form part of an evolutionary stable strategy by this mechanism because constitutive defended strategists could readily invade an inducible population and an inducible mutant could not increase in frequency in a population of constitutive defended plants.

Induction may be more fit than constitutive defence when plants are faced with herbivore specialisation. Adapted insects can be a significant cost to plants (Islam & Crawley 1983; Giamoustaris & Mithner 1995; Wise & Sacchi 1996) because they are not deterred from feeding by plant resistance and may even use it as an attractant. Karban and Myers (1989) proposed that induction could solve this problem simply by changing the plant's phenotype to any other state. In this hypothesis it is argued that unidirectional induction, in which the induced state is always the 'defended' state, will be disadvantaged if there are high densities of adapted herbivores because induction makes these plants more susceptible to attack. Provided herbivore adaptation to the defended phenotype carries a fitness trade-off when on the undefended phenotype such that less damage to undefended plants results, changing randomly to any phenotype will be a better strategy than directional induction as it is less likely that the plant will adopt the strategy to which the adapted herbivore can efficiently feed on. Constitutive undefended strategists will be favoured if the adapted herbivore density gets too high or there is a high physiological cost to defence (Adler & Karban 1994). Karban and Baldwin (1997) have also argued that this 'moving target' mechanism may also decrease the rate of herbivore counter-adaptation because the selection pressure for any particular herbivore phenotype is never constant. The difficulty with the model is that most induced responses seem to be directional because the damage induced change in the plant phenotype is restored to the original state in the absence of further herbivory (*e.g.* Dewick 1975;

Daddona *et al.* 1976; Edwards *et al.* 1985; Bryant *et al.* 1991; Tallamy & McCloud 1991; Gershenzon 1994; Karban & Baldwin 1997; Baldwin 1998; Young & Okello 1998). Furthermore, some herbivores seem to benefit from variation in their diets (Bernays *et al.* 1992) or experience no trade-off on undefended hosts when adapted to defended hosts (Scriber 1978; Anderson & Alborn 1999), and so it seems unlikely that this mechanism is very widespread.

The final hypothesis also makes a case for the role of specialised insects in the evolution of plant defence strategies. This hypothesis contends that the intermittent expression of defensive characters may reduce damage by adapted herbivores by not expressing resistance traits that attract them (Karbon & Baldwin 1997). Implicit in this escape hypothesis is the need to balance effective defence against non-adapted herbivores with the risk of attracting specialists. For this reason the hypothesis is not concerned with induced responses that have no resistance effect, such as when previous defoliation increases palatability by promoting growth flushes (Webb & Moran 1978; Craig *et al.* 1986; Pullin 1987; Potter & Redmond 1989; Messina *et al.* 1993), or increases the nutritional value of the plant in the absence of a decrease in palatability to other herbivores (Kielkiewicz 1988; English-Loeb & Karban 1991). There is considerable evidence that adapted herbivores use plant resistance traits as cues for identifying their host plants (Ehrlich & Raven 1964; Nault & Steyer 1972; Renwick & Chew 1994). However in many examples in which the induced resistance state is known to be attractive (Lindstrom *et al.* 1990; Tallamy & McCloud 1991; Landolt 1993; Uematsu & Sakanoshita 1993; Pivnick *et al.* 1994; Baur *et al.* 1996; Anderson & Alborn 1999), the herbivores are also able to feed, or will oviposit on the uninduced state as well, and so escape is not simply a matter of adopting the undefended phenotype. If the adapted herbivore feeds too heavily on undefended plants we might expect there to be a higher fitness pay-off for those plants adopting the defended phenotype. Similarly, if defended plants receive higher levels of herbivory, the undefended phenotype will be favoured provided that the cost of non-adapted herbivores is not too large. A critical region is present, in which both conditions favour induction, if the induced response is effective at reducing herbivory by non-adapted herbivores. Whether the induced strategists lie in this critical region depends on how the herbivores are distributed between the defensive phenotypes. One factor that has been identified as affecting the distribution of herbivore feeding between plants is host-plant frequency (Cottam 1985). It is the role of frequency-dependent feeding in the evolution of plant defence strategies that this study will investigate.

### ***FREQUENCY-DEPENDENT ESCAPE AND INSECT HOST CHOICE***

We know that the early season defensive state of a plant relative to the other plants in the population can affect the late season probability of herbivory (*e.g.* Wallner & Walton 1979; Raupp & Denno 1984; Haukioja & Neuvonen 1985; Tallamy 1985; Baldwin 1988; Karban 1993; Leather 1993; Giamoustaris & Mithner 1995; Wold & Marquis 1997). The ways in which phenotype variation within an inducible population can be maintained have also been identified. Typically, the trend to uniform induction may be countered by the relaxation of induced individuals and new plants entering the population (Edelstein-Keshet & Rausher 1989). While there is disagreement among the modellers as to whether these undefended individuals can be maintained in the presence of herbivores (Edelstein-Keshet & Rausher 1989; Underwood 1999), the early season experiments indicate that they may if herbivore densities, and the corresponding incidence of damage, are sufficiently low. Significantly for the escape hypothesis, no attempts have been made to assess the consequences for the evolution of plant defence strategies of variation in the relative frequency of the induced state and the ensuing host choice behaviour of adapted herbivores. There is a need to understand how host choice varies with induced phenotype frequency in both a mechanistic, and a functional sense. This study will focus on maternal host choice in insects with relatively immobile offspring.

### **Motivation models**

During the last two decades, our understanding of oviposition acceptance behaviour has been heavily influenced by the motivation state paradigm (Dethier 1982; Singer 1982; Miller & Strickler 1984; Courtney *et al.* 1989; Jaenike & Papaj 1992; Mangel 1993; Mayhew 1997). Principally, motivation state models assume that a lack of host discrimination is a passive consequence of the failure to find the more preferred host. They predict that in the absence of egg-laying, physiological changes such as increasing egg-load and other unspecified mechanisms trigger acceptance of lower quality hosts (Singer *et al.* 1992). Central to this is the concept that the acceptance of a host plant may be ranked in time relative to other plants according to its suitability for larval development (Mayhew 1997). It is still uncertain however, if there is in fact a reliable correlation between oviposition preference and host suitability (Thompson 1988; Thompson & Pellmyr 1991). There is also an increasing acceptance of the role of learning in motivation models (Jaenike & Papaj 1992; Mangel 1993), reflecting the growing literature on the subject (reviewed Papaj & Prokopy 1989;

Szentesi & Jermy 1990). Despite this, learning is generally presumed to be unnecessary for acceptance to occur.

Evidence cited in support of the motivation models has failed to demonstrate an effect of increasing egg-load on decreasing discrimination between host plants. A number of studies show an increase in oviposition acceptance and/or the numbers of eggs laid with increasing egg-load (Fitt 1986; Courtney & Chen 1988; Courtney *et al.* 1989; Odendaal & Rausher 1990; Horton & Krysan 1991; Rosenheim & Rosen 1991; but see comments regarding methodology in Minkenberg *et al.* 1992 and Mayhew 1997), however many of these have not tested for changes in discrimination. Of those studies that have, most have methodological problems. Findings of decreased discrimination with egg-load have been confounded with egg-spacing behaviour (Wasserman & Futuyma 1981) and feeding experience (Courtney *et al.* 1989). Fitt (1986) found no relationship between egg-load and discrimination in *Dacus tyroni* (Diptera: Tephritidae), but his experiment was confounded with adult experience. In addition, the usefulness of Fitt's results may also be limited by the failure of two-way choice experiments to separate pre- and post-alighting discrimination, which may not be correlated (Papaj & Rausher 1983). In a study that manipulated egg-load in *Ceratitis capitata* flies by altering the protein content in the larval diet, Prokopy *et al.* (1994) found that discrimination increased with egg-load when the flies were tested in large cages. However, both test hosts were equally acceptable when tested in small cages regardless of egg-load, perhaps indicating an effect of egg-load on search intensity in the larger cages.

In the face of such equivocal evidence for the role of egg-load in discrimination between host plants, it is clear that further study is required. It is important to resolve the significance of general physiological factors such as egg-load and more specific processes like learning on the host choice process as each may have different outcomes for the patterns of host acceptance. Acceptance models based primarily on general motivational factors predict that low quality hosts will only be accepted in the absence of encounters with better hosts (Courtney *et al.* 1989). By contrast, the probability of expressing a learned response is dependent on the encounter frequency with the stimulus (Abramson 1994). Therefore a model based on learning predicts that a frequently encountered low quality host can become acceptable before a higher quality host that is encountered rarely (Jaenike & Papaj 1992).

### ***THE STUDY AIMS***

This dissertation investigates the role of frequency-dependent herbivory by adapted insects in the evolution of plant defence strategies. Specifically, the study:

- tests the motivation state models by determining the roles of egg-load and learning in the host acceptance process;
- examines the effects of variation in the relative frequency of induced phenotypes within a patch, and larval experience, on the host choice behaviour of the diamondback moth;
- formally models the consequences of frequency-dependent acceptance by adapted herbivores for the evolution of constitutive and inducible plant defence strategies.

### ***THE BIOLOGY OF THE STUDY ORGANISM***

All experiments were conducted using the diamondback moth (*Plutella xylostella* L. (Lepidoptera: Yponomeutidae)).

The diamondback moth is approximately 9 mm long and is characterised by the cream coloured diamond pattern observed on the folded wings, particularly in males. The larvae of the moth feed primarily on plants that are members of the Brassicaceae (Talekar & Shelton 1993). The mustard oils and their glucosides that are found in members of this plant family act as phagostimulants to the larvae (Talekar & Shelton 1993) and as oviposition attractants to the adult females (Gupta & Thorsteinson 1960; Pivnick *et al.* 1994).

A single female moth can lay up to 450 eggs during her lifetime, which in captivity is generally 20 - 28 days. The eggs are laid either singly, or in plates of 30 or more, and are typically laid on the lower stem, cotyledons, or the base of the main veins of leaves. Females are apparently not deterred from ovipositing by the presence of conspecific eggs (Groeters *et al.* 1992). First instar larvae burrow into the leaves, typically emerging during the second instar to feed on the leaf surface. There are four larval instars in addition to a pre-pupal stage (Robertson 1939). In captivity, at temperatures above 16 °C, a complete life cycle from egg to adult takes approximately 26 - 28 days.

Diamondback moths are a cosmopolitan pest of Cruciferous crops (Talekar & Shelton 1993). In New Zealand, diamondback moths suffer high mortality due to the larval parasitoid *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and the pupal parasitoid *Diadromus collaris* (Hymenoptera: Ichneumonidae) (Beck & Cameron 1990). In addition, the fungal

pathogen *Entomophthora sphaerosperma* can cause considerable mortality in humid conditions (Robertson 1939). Consequently, crop damage in New Zealand by diamondback moths appears to be less severe than in parts of Asian, although minor cosmetic damage can be a problem (Beck & Cameron 1990).

### **PREFACE TO THE CHAPTERS**

**Chapter 2** examines the roles of learning and egg-load in the oviposition acceptance behaviour of the diamondback moth. It specifically tests the hypothesis that prior experience with a host plant increases acceptance in a host-specific manner. The effects of increasing egg-load and the non-specific 'time since last oviposition' on discrimination between plants are also examined by depriving moths of host plants. The results are discussed and a new model of oviposition preference proposed.

**Chapter 3** examines the oviposition behaviour of diamondback moth within patches comprising different mixtures of damaged and undamaged *Brassica* plants (*B. oleracea* L. and *B. napus* L.). Damage has been previously shown to cause an induced response in brassicas and the associated odours are preferentially attractive to diamondback moths (Pivnick *et al.* 1994). The experiments test whether the oviposition response of the moths within a patch is dependent on the relative frequency of induced hosts. In addition, this chapter also investigates larval experience as a possible mechanism to explain preference for induced plants in diamondback moth.

**Chapter 4** formally models a defensive strategy set comprising constitutively undefended, constitutively defended and inducible strategists in the presence of non-adapted and adapted, or partially adapted herbivores. It is assumed that adapted herbivores are distributed between plants of different phenotype as a function of the relative frequency of the host phenotypes present. The model is analysed using an adaptation of the evolutionary stable strategy (ESS) framework as proposed by Maynard-Smith (1982). The model examines the conditions under which each of these strategies may form pure ESS's, or evolutionary stable states in conjunction with other strategies, in the absence of physiological costs to resistance. Special attention is paid to the role of phenotype variation and its interaction with insect host choice behaviour to determine the relative cost of herbivory to each plant strategy.



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**Chapter 5** discusses the study's findings with a particular emphasis on the adapted herbivore escape hypothesis. Attention is drawn to the effects of adapted insect host choice on the stability of the defence strategies and its implications for the experimental testing of the fitness benefits of induced resistance. Further research to test the adapted herbivore escape hypothesis is proposed.

## CHAPTER 2

# MOTIVATION STATE MODELS AND OVIPOSITION ACCEPTANCE BEHAVIOUR IN THE DIAMONDBACK MOTH

### *INTRODUCTION*

The fitness of phytophagous insects with immobile offspring depends on females choosing the most suitable oviposition site for larval survival and development. But if females set too high a standard during site selection, they run the risk of becoming time-limited whereby the full complement of eggs is not laid by the time of death. The decision to accept or reject a particular host plant that maximises fitness depends on the probability of encountering better hosts in the remaining available search time (Jaenike 1978). To determine the probability of encountering a better host requires information about habitat composition. If the habitat composition is relatively predictable between generations then this information could be permanently encoded in the genome and expressed through some form of innate evaluation process (Stephens 1993). However, plant populations generally vary considerably over both a temporal and spatial scale (Crawley 1990) and even a small change in host-plant predictability will make a fixed acceptance response unreliable for determining the best hosts to accept (Stephens 1993). More variable environments require some form of sampling to gather this information.

A class of models, referred to here as 'motivational state models', has been developed to explain how females evaluate habitat information during searching for host-plants. These models begin with the premise that there is an internal motivational state, or states, that determine when a particular host will be accepted for oviposition. This motivational state changes with internal physiological changes that measure the perceived risk of time limitation. Motivation models imply that insects rank hosts according to the relative time during searching at which they are accepted. High-ranking hosts will be accepted when the perceived risk of time limitation is much smaller than that at which lower ranked hosts become acceptable.

The origins of motivation models lie with Dethier (1982) and Singer (1982) who suggested an association between the internal physiological state of the insect and discrimination between host-plants. Miller and Strickler (1984) elaborated on their ideas to formulate the

Rolling Fulcrum Model. These early models assume a single motivation state determined by the internal physiological condition of the insect. Changes in motivation have been attributed to changes in female egg-load or time since last oviposition, but the exact physiological mechanism remains unknown (Singer *et al.* 1992). Increased acceptance through host-specific experience (Jaenike 1983; Rausher 1983b; Prokopy *et al.* 1993, 1994a; Anderson *et al.* 1995; Cunningham *et al.* 1998) creates a problem for these models in that the changes in motivation must be a response to external host cues.

One approach to incorporating learning into motivational state models can be found in the hierarchy threshold model (Courtney *et al.* 1989; Courtney & Kibota 1990). In this model, experience on a host increases the probability of accepting that host and all those ranked above it. Hence, it is argued that learning acts on motivation in a non-specific manner and has been used as a possible explanation for cross induction of preference (Courtney *et al.* 1989) in which experience on one species increases acceptance of another species. The hierarchy threshold model does not predict reversals in preference rank with experience such as have been shown for *Drosophila melanogaster* (Jaenike 1983) and the blueberry maggot fly, *Rhagoletis mendax* (Prokopy *et al.* 1993).

Models in which females show species-specific motivation can explain reversals in preference rank (Jaenike & Papaj 1992; Mangel 1993). These models assume learning causes changes to species-specific motivations that increase the likelihood of laying on one host relative to another, regardless of preference rank. Thus preference rank can change depending on previous experience. The frequency of experience and the intensity of the stimulus both contribute to an increased probability that the host will be accepted (Jaenike & Papaj 1992). As with other motivational threshold models, in the absence of any experience on hosts, females will become less discriminating with time or increasing female egg-load.

The link between egg-load, or time since last oviposition, and discrimination has not been adequately tested. It seems quite clear that high egg-load increases the number of eggs laid in each clutch or per unit time (Fitt 1986; Odendaal & Rausher 1990; Rosenheim & Rosen 1991). In general, high egg-loads also result in an increase in the probability of host acceptance (Courtney & Chen 1988; Courtney *et al.* 1989; Odendaal & Rausher 1990; Horton & Krysan 1991; Prokopy *et al.* 1994b) but fewer studies have tested for changes in discrimination. In one of these studies, Wasserman and Futuyma (1981) found that the number of eggs laid by the southern cowpea weevil, *Callosobruchus maculatus* was

correlated with increased acceptance of the less preferred host in two-way choice tests. The authors attributed this to avoidance of an oviposition deterring pheromone because of the limited availability of oviposition sites rather than egg-load dependent discrimination. Fitt (1986) detected no decrease in discrimination with increasing egg-load in *Dacus tyroni* (Diptera: Tephritidae) in two-way choice tests. This result must be treated with caution however, as the acceptability of the preferred host may have been inflated regardless of egg-load, due to exposure to this host prior to testing. In agreement with the motivation state models, a further study found that the difference in the percentage of the total egg complement laid on the two test substrates by *Drosophila busckii* (Diptera) was maximised at intermediate levels of egg-load (Courtney *et al.* 1989). However, feeding on the substrates during testing may have affected the relative maturation rates of the eggs during the test period leaving more eggs to be laid by flies on the preferred substrate. Furthermore, it is not possible to distinguish between changes in the proportion of eggs laid that were due to the effects of clutch size, and those due to changes in rates of acceptance. In the final study, Prokopy *et al.* (1994b) found that high egg-load in *Ceratitis capitata* (Diptera) increased acceptance of the preferred host relative to the non-preferred host in large cages, but had no effect in small cages. Thus there is little evidence for the link between egg-load and discrimination between host species as predicted by motivation state models.

Ambiguity in determining the respective roles of host experience and egg-load in host finding behaviour has also arisen because these factors are confounded in most of the studies testing for egg-load effects (Minkenberg *et al.* 1992). Other studies that correlate the time since last oviposition with acceptance following repeat presentations of hosts (*e.g.* Singer 1982, 1983) could also be explained by learning, because the females are effectively being given a training regime during testing.

This chapter sets out to test the motivational state models by looking at the effects of learning and host-deprivation on host acceptance by ovipositing diamondback moths (*Plutella xylostella* L. (Lepidoptera: Yponomeutidae)). The diamondback moth has a broad host range within the Cruciferae (Talekar & Shelton 1993) and learning has been shown to affect larval food acceptance (Lee & Tseng 1993). *Plutella* also continuously matures eggs and is therefore physiologically suited to the manipulation of egg-load by host deprivation to test for an effect on host acceptance. I tested whether learning in adult diamondback moths is host species-specific or also results in increased acceptance of all higher ranked hosts as predicted by the hierarchy threshold model. I specifically tested for non-associative learning in which

the repeated presentation of a stimulus results in an alteration of the probability or strength of the response to the stimulus (Abramson 1994). Learning in response to a novel host must be non-associative, at least initially (Szentesi & Jermy 1990), as the host cannot elicit the oviposition response to act as an unconditioned stimulus before the host is actually acceptable. I also tested the prediction of motivation state models that increasing egg-load or time since last oviposition, as manipulated by host deprivation, results in a reduction in discrimination between host-plants. Under these models, a lower ranked host should be more likely to be accepted for oviposition by the insects relative to a higher ranked host if the insects have been deprived of hosts for a period of time. Furthermore, if sufficient time has elapsed for both host-plants to have been equally likely to have been accepted, then under the motivation state models, depriving insects of host-plants for longer than this period should result in a continuing lack of discrimination.

## **METHODS**

Diamondback moth larvae were reared in the University of Canterbury glasshouse on four week old potted rape plants (*Brassica napus* L. cv. "rangi") at a density of six larvae per plant. The larvae came from a stock culture maintained on rape for two years.

All experiments were carried out at  $50 \pm 10\%$  RH at a temperature of  $21 \pm 1$  °C. A single 400W sodium lamp on a 16:8 hr on/off cycle provided the lighting. The beginning of the scotophase was synchronised to the natural daylight finish as experienced during larval rearing. Moths were housed in 20 cm high four litre black plastic cages during the experiments. These cages had fine mesh on one side and on most of the roof. The cages were large enough to allow short distance free flight but sufficiently small that there was little chance of a searching insect not encountering the host-plant. Thus while not strictly measuring post-alighting discrimination, the effect of pre-alighting orientation would have been minimal. All rape, cabbage (*Brassica oleracea* L. cv. "golden acre merit") and cress (*Nasturtium officinale* L. Br. cv. "salad curled") plants were grown in fertilised potting mix in the glasshouse.

### **The Specificity of Non-Associative Learning**

One female and three male moths were placed in each of 40 cages with an 18 day old rape plant and a 10% sucrose solution cotton wick feeder. Rape plants were removed after night three and the eggs counted. Only those insects that had laid at least 20 eggs during this

period, and were in good physical condition, were used in the test procedure. On night four, females had no access to hosts to limit the effects of prior experience from exposure to rape and to ensure that willingness to oviposit was high. At the commencement of the scotophase on night five, females were given either a pot containing potting mix without a plant or one containing potting mix and a 14 to 18 day old cabbage plant. Plants were removed and checked for eggs after 15 minutes. Only moths that did not lay during this training period were included in the subsequent analysis. This restriction served to reduce the possibility of differences in egg-load between treatments affecting acceptance. In addition, it also ruled out the possibility that either the act of egg-laying, or the already acceptable plant, could act as an unconditioned stimulus that could be associated with some other part of the test procedure to subsequently trigger oviposition. Females were then given no access to plants for 10 minutes in order to minimise the possible effects of effector fatigue and sensory adaptation on testing (Abramson 1994). Either a 14 to 18 day old cress or cabbage was then given to females for 15 minutes in a no choice situation. The training and test periods were chosen to be sufficiently short that untrained insects rarely accepted cabbage. To detect learning however, the periods had to be long enough such that the combined length of the training and testing periods was equal to, or longer than, the length of time required for acceptance of cabbage to be measurably greater than during either period alone. Eggs were counted and acceptance was deemed to have occurred if at least one egg was laid on the plant during the test period. Females were tested once and then discarded. Five separate runs of this experiment were carried out.

### The Effect of Host Deprivation on Acceptance

One female and three male diamondback moths were placed in the cages as before with a sucrose solution feeder. Each moth had free access to rape on nights one to five following emergence. On nights six and seven, one group was deprived of all hosts and the other group continued to have access to rape. The test period for host deprived and non-deprived insects commenced at the beginning of the scotophase on night eight. Rape plants in the non-deprived group were removed eight hours prior to testing to minimise the effects of previous experience on the test procedure. Females were tested for acceptance of either three week old cress or cabbage in no choice trials. Only those females that laid eggs during the last night in which they had access to rape were used during testing. This gives a definite night on which females last laid eggs. Test plants were checked for eggs following an initial 20 minute test period. Eggs were not removed. Females were then allowed to oviposit for the remainder of the night before eggs were again counted. Acceptance of the test plant was considered to

have occurred if at least one egg was laid on it. Four separate runs of the experiment were carried out.

A further group of host-deprived and non-deprived females was dissected at the beginning of night eight in order to confirm that deprived moths had a higher egg-load than non-deprived moths.

### Long-term experience and the inhibition of cabbage acceptability

If long-term experience is important in determining acceptance, it is possible that previous experience on rape could affect acceptance during the deprivation experiment. This previous experience could affect deprivation treatments unequally as the experience is more recent in non-deprived insects, or it may inhibit the ability of insects to respond to increases in egg-load. Recent experience of cabbage was examined in the learning specificity experiment, and so this experiment tested whether previous long-term exposure to rape or cabbage affected subsequent acceptance of cabbage.

One newly hatched female and four males were placed in the four litre plastic cages as before. Each female was randomly assigned to one of three treatment groups. For the first four nights following emergence, groups one and two were given access to a single 16 to 18 day old rape plant and group three received a similarly aged cabbage. On night five, group one continued to receive rape while groups two and three received cabbage (Table 2.1). All groups received new plants on night five. The treatment applied to group two moths was included to distinguish between an increase in acceptance of cabbage by group three moths due to long-term cabbage exposure, and a decrease due to experience on rape. The training plants were removed one hour before testing on night six. Moths up until this time were fairly inactive and had probably had little contact with the training plant since the previous night. Only those moths that laid more than 20 eggs during nights one to four were tested. Failure to

**Table 2.1.** *The experimental design for the long-term experience and inhibition experiment.*

Group	Host-plant nights 1-4	Host-plant night 5.
One	Rape	Rape
Two	Rape	Cabbage
Three	Cabbage	Cabbage

lay more than 20 eggs was not associated with a particular treatment (Chi-squared test,  $\chi^2_1 = 0.178$ ,  $p = 0.67$ ). The test procedure consisted of giving all groups a 16 day old cabbage plant for a period of 45 minutes at the beginning of the scotophase and recording acceptance. Acceptance was considered to have occurred if at least one egg was laid on a cabbage test-plant. The experiment was run three times.

## Analysis

All pair-wise comparisons of acceptance counts were made using two-sided  $\chi^2$  tests under the expectation that there was no difference in acceptance counts between conditions. Analysis of the effects of deprivation on the host acceptance data for the 20 minute test period used a generalised linear model with binomial error and logit link function, performed using S-Plus (Version 3.3, Mathsoft Inc. 1995). Logit models are appropriate when looking at the effect of categorical variables on a dichotomous response variable (Fienberg 1980) such as acceptance. The probability structure of cross-classified data gives rise to multiplicative effects of the parameters on the response variable. The logit link function linearises the relationship between the parameters and the estimate of the expected value of the response variable (McCullagh & Nelder 1989). The GLM modelled the effects of run, host deprivation and test plant species on acceptance. Run was included as a blocking variable. A GLM with Poisson error and log link function was used to analyse the egg count data for the 20 minute test data. As before, host deprivation and test plant species were used as explanatory variables and run served as a block. The significance of the explanatory variables in the GLMs were tested using two-sided  $\chi^2$  tests. When the data were over-dispersed, as indicated by a departure from 1 of the ratio of residual degrees freedom to residual deviance, then the significance was assessed using  $F$  tests (Crawley 1993).

## RESULTS

### Specificity of learning

Training on cabbage produced a host-specific increase in acceptance of cabbage (Table 2.2). Untrained moths accepted cress more often than cabbage (Chi-squared test,  $\chi^2_1 = 10.15$ ,  $p = 0.0014$ ). However, when moths were given fifteen minutes prior experience on the less preferred host cabbage, there was no difference in the rate of acceptance between cress and cabbage ( $\chi^2_1 = 0.17$ ,  $p = 0.68$ ). The training effect was host-specific as training on cabbage



**Table 2.2.** *Acceptance counts for the effect of cabbage training on acceptance of cabbage or cress. Acceptance values in columns or rows followed by the same letter subscript are not significantly different at  $\alpha = 0.05$  using  $\chi^2$  tests. See the text for details of tests.*

Host tested	No training.		Trained on Cabbage	
	<i>N</i>	% accepting host	<i>N</i>	% accepting host
Cabbage	31	16.1 <sub>a</sub>	26	53.8 <sub>b</sub>
Cress	31	54.8 <sub>b</sub>	27	48.1 <sub>b</sub>

had no effect on acceptance of cress ( $\chi^2_1 = 0.25$ ,  $p = 0.61$ ) but did increase acceptance of cabbage ( $\chi^2_1 = 9.05$ ,  $p = 0.0026$ ). During the training period no females laid on empty pots but thirty-one females laid on cabbage during training. It is possible that the females who laid during training were the most motivated to lay. Removing these females from the analysis would then bias the results away from detecting a general increase in acceptance with training. A general increase in acceptance would be indicated by an increase in the acceptance of cress with training on cabbage. If those that laid during training and were tested on cress are included in the analysis there was still no effect of training on acceptance of cress ( $\chi^2_1 = 0.013$ ,  $p = 0.91$ ).

### Host Deprivation and Acceptance

Moths deprived of hosts for two days prior to testing contained more eggs than moths with unrestricted access to hosts (mean  $\pm$  SE =  $81.5 \pm 10.6$  and  $35.8 \pm 6.5$  respectively; one tailed t-test,  $t_{24} = 3.79$ ,  $p = 0.0004$ ). Host deprivation is therefore a useful technique to test for the effects of egg-load, as well as the time since last oviposition, on discrimination between hosts.

The acceptance data for the effects of host deprivation are given in Table 2.3. Non-deprived moths were equally likely to accept cabbage or cress after one night (Chi-squared test,  $\chi^2_1 = 0.42$ ,  $p = 0.52$ ). This measured a maximum time after which both hosts were acceptable, rather than measuring the relative attractiveness of the plants to the moths, as non-deprived females preferred cress over cabbage during the first 20 minutes of testing ( $\chi^2_1 = 4.53$ ,  $p = 0.033$ ). If discrimination is primarily controlled by egg-load or time since last oviposition, then moths deprived of hosts for two nights should show no preference for cabbage or cress

**Table 2.3.** *Acceptance and egg count data for host deprived and non-deprived moths tested on cabbage and cress. The number of eggs that were laid is given as the mean  $\pm$  SE.*

Test Period	Deprivation	Tested on Cabbage			Tested on Cress		
		<i>N</i>	% accepting host	Mean no. eggs laid	<i>N</i>	% accepting host	Mean no. eggs laid
20 min.	Non-deprived	27	11.1	$0.33 \pm 0.21$	25	36.0	$1.2 \pm 0.46$
	Deprived	27	29.6	$0.59 \pm 0.21$	26	69.2	$2.81 \pm 0.74$
1 night	Non-deprived	27	81.5		25	88.0	

during the first 20 minutes of testing since the probability of acceptance by non-deprived females was equal after one night. Contrary to this expectation, deprived females were more than twice as likely to accept cress as cabbage during the first 20 minutes of testing ( $\chi^2_1 = 8.31, p = 0.004$ ).

An effect of egg-load on discrimination can also be detected if deprivation changes the probability of accepting a host relative to the other host during the 20 minute test period. During this test period, cress was accepted more than cabbage regardless of deprivation (binomial GLM,  $\chi^2_1 = 13.61, p = 0.0002$ ). Deprivation increased the general likelihood of accepting a host ( $\chi^2_1 = 8.94, p = 0.0028$ ) but did not increase the likelihood that cabbage would be accepted relative to cress (deprivation x test plant interaction;  $\chi^2_1 = 0.02, p = 0.90$ ). Analysis of the number of eggs laid during the 20 minute test period also suggests that there was no decrease in discrimination with increasing egg-load (Table 2.3). During this test period significantly more eggs were laid on cress than cabbage (Poisson GLM,  $F_{1,98} = 17.31, p < 0.0001$ ) and more eggs were laid by deprived moths than those that were not deprived ( $F_{1,98} = 6.03, p = 0.016$ ). As before, the effect of deprivation was not influenced by the test plant (deprivation x test plant interaction,  $F_{1,98} = 0.218, p = 0.64$ ) indicating no change in discrimination.

### Inhibition of acceptance by long-term experience

There was no evidence that prior exposure to rape decreased acceptance of cabbage (Table 2.4). Moths given five days prior experience on rape were not less likely to accept cabbage during testing than those given cabbage on the fifth night or those only exposed to cabbage (Chi-squared test,  $\chi^2_2 = 0.378$ ,  $p = 0.83$ ). Consequently, the result in the deprivation experiment cannot be explained by inhibition of cabbage acceptance by previous exposure to rape. The results also demonstrate that long-term experience on cabbage does not affect subsequent acceptance of cabbage, indicating that the effects of experience illustrated in the learning experiment appear to be short-term.

**Table 2.4.** *Long-term previous host-plant experience and its effect on acceptance of a cabbage test plant.*

Host-plant nights 1-4	Host-plant night 5.	% accepting test plant	<i>N</i>
Rape	Rape	45.8	24
Rape	Cabbage	47.8	23
Cabbage	Cabbage	54.5	22

### DISCUSSION

Recent experience of cabbage increased oviposition acceptance of cabbage, but not cress. The data seem to indicate that experience acts in a species-specific manner, although an alternative interpretation is possible. Potentially, this effect could be due to a more general effect of plant experience, but that this is not evident because acceptance of cress has in some way been inhibited. There are two possible ways in which acceptance of cress could have been inhibited. The first is that the pre-testing experience of rape on days 1-4 could have made the insects unresponsive to cress. I believe this to be unlikely because the absence of a long-term effect of experience, despite the obvious effect of short-term experience, suggests that experience gained in the distant past is irrelevant to the oviposition choices being made by diamondback moths. Great care was taken to ensure that moths did not have recent experience of rape by limiting the last exposure to host plants to two nights prior to testing. The second reason why the absence of a more general effect of cabbage experience on acceptance of cress could have been obscured is if the maximum rate of acceptance for any

host plant were equal to the acceptance rate of cress. Therefore a general effect would not be observed because the maximum number of moths that were motivated to lay did so, whether they had had previous experience of cabbage or not. We might expect such a constraint if, for example, the maximum probability of laying was constrained to be less than 1 by variation between moths in the maturation rate of eggs, or in the time at which searching for hosts commenced. This possibility could be unequivocally ruled out if training on cress increased the acceptance rate of cress. While I do not have these data, two factors count against this explanation. The first is that after a single night's oviposition in the deprivation experiment, 88% of moths laid eggs on cress. This indicates that the potential response rate is much higher than the 51% response rate to cress averaged across both treatments in the learning experiment. The second point is made by examining the acceptance rates for moths that were tested on cress and trained on cabbage, including those moths that laid eggs during training. 67.4% of these moths oviposited on either cabbage during training, cress during testing, or both. Oviposition during training did not affect acceptance of the test plant, so it seems that those moths that only laid during training were probably still motivated to oviposit provided a suitable host was available. Therefore the 48.1% recorded accepting cress following training on cabbage is probably an underestimate of the true proportion of insects that could have potentially laid eggs. When all these factors are considered, the most probable explanation for these data is that experience increases acceptance in a host-specific manner.

Host-specific acceptance strongly suggests separate 'motivations' to oviposit in the sense of Jaenike and Papaj (1992). By Courtney *et al.*'s definition (Courtney *et al.* 1989), cabbage is ranked below cress because cabbage was accepted less frequently than cress. I have already discarded the possibility that experience on rape could have effected this estimate, but even if it had these data still invalidate Courtney *et al.*'s model. If in reality cress and cabbage were equally ranked, then experience on cabbage should have increased acceptance of both hosts under the hierarchy threshold model. If cabbage were ranked higher than cress then we should not have observed lower acceptance of cabbage with *any* kind of experience. Hence, if 'motivation' were a general moving threshold as envisaged by Courtney *et al.* (1989) and Singer *et al.* (1992), then training on cabbage should have increased acceptance of cress – clearly it did not (Table 2.2). Host specific 'motivation' implies that the concept of host ranking is context sensitive and not an intrinsic property of the female. Hence, the usefulness of ranking as a predictor of behaviour is limited by the need to measure it relative to the particular host composition of the habitat.

The effect of recent experience is most consistent with non-associative learning. Insects were naive with respect to cabbage and cress prior to training, and insects were rejected from consideration if they laid eggs during training. Therefore the training stimuli, or the actual act of laying an egg, could not have acted as an unconditioned stimulus because no unconditioned oviposition response was elicited. Bernays and Weiss (1996) suggest that in caterpillars, non-associative learning is most likely to involve habituation to individual deterrents. If this is the case, then insects must evaluate each host-plant cue independently. However, the indications are that individual cues interact non-additively to influence oviposition choice in diamondback moth. Spencer (1996) has found that leaf waxes enhance diamondback moth attraction to the plant compound sinigrin even though moths do not respond to waxes on their own. If single cues are shared between species then habituation or sensitisation to a particular cue is less likely to produce a species-specific change in acceptance with experience than if many cues are used. All species that share the particular cue will also become more acceptable. The alternative is that insects become sensitised to the whole cue profile. Bernays and Weiss (1996) objected to a role for sensitisation in caterpillar feeding because they felt it was too short-term to be important, however the duration of the behavioural modification reported in the present study is well within the range for the response periods reported by Carew (1984). More work is required to differentiate between these two alternatives, and would need to focus on the effects of experience on the response to host-plant compounds, both singly and in combination.

Host deprivation affected acceptance independently of the host-specific cues that were involved in the learning effect. Deprivation increased egg-load and time since last oviposition, both of which are thought to decrease discrimination in motivational state models (Singer 1982; Miller & Strickler 1984; Courtney *et al.* 1989; Courtney & Kibota 1990; Jaenike & Papaj 1992). This study has found no evidence for a decrease in discrimination with increasing deprivation. The absence of a relationship between discrimination and deprivation was manifest in two ways. In the first instance only one night was required for both hosts to be accepted with the same probability. If egg-load or time since last oviposition controls discrimination, depriving insects of hosts for longer than this period should also have resulted in no preference for cress. Instead, a strong preference for cress was still recorded for deprived moths tested during the first 20 minutes of the scotophase (Table 2.3). It could be argued that the measurement of time after which both hosts were equally acceptable was flawed, because if learning is also involved, then the time at which equal acceptance was measured would have been sooner than if deprivation effects

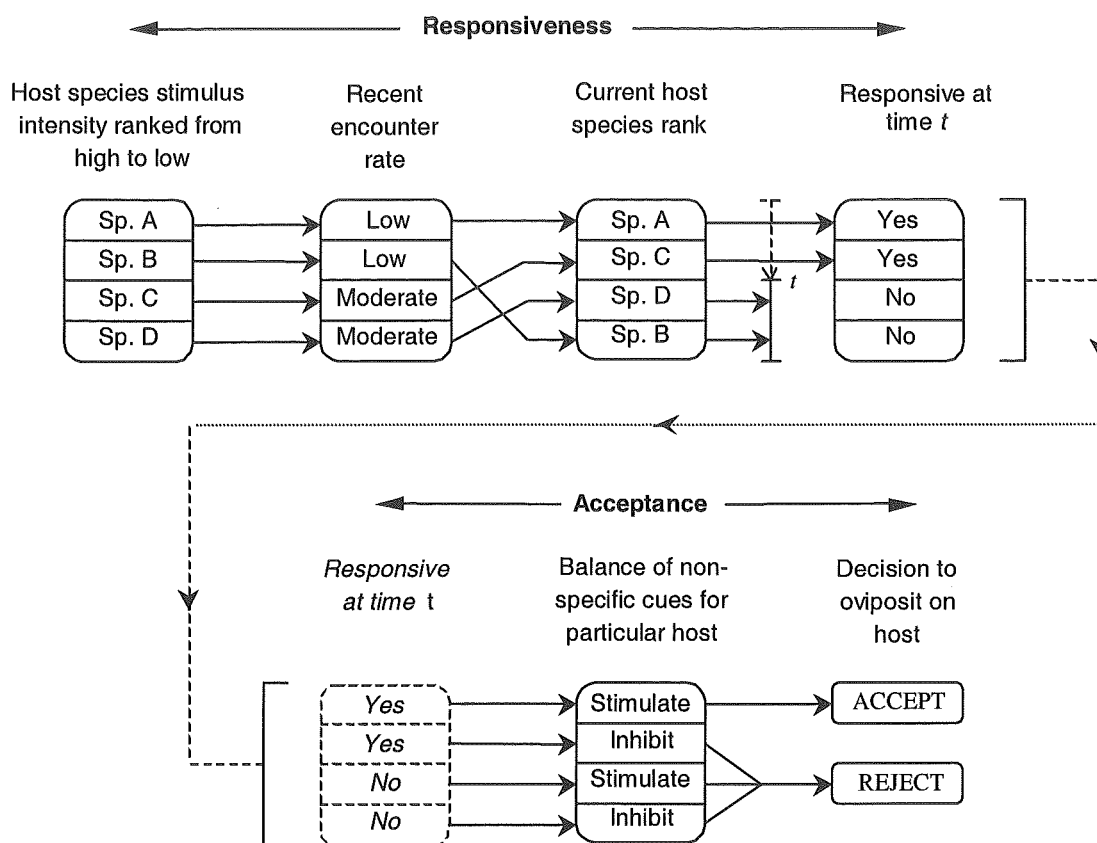
alone were responsible. If valid, this argument would also suggest that deprivation was unimportant in determining discrimination. If two nights deprivation produced no decrease in discrimination during the first 20 minutes of testing, yet one night's experience and accumulated egg-load produces no discrimination at all, then deprivation at best could only have had a very minor effect.

The analysis of the 20 minute test period data reveals the second way in which an effect of deprivation on discrimination was indicated. Cabbage was not more likely to be accepted relative to cress with increasing deprivation. Rather, there was a small non-specific increase in acceptance regardless of host-plant. I believe that an increase in search intensity resulting from higher egg-load (Minkenberg *et al.* 1992) can be ruled out as an explanation for this increased acceptance. An increase in search intensity would increase the encounter rate with the host-plant. As non-associative learning is a function of the interaction between encounter frequency and stimulus intensity (Petrinovich 1984), a change in search intensity would cause species-specific changes in acceptance rather than the general change observed here. Another possibility is that experience on rape prior to testing could have influenced acceptance of cabbage or cress, such that a change in discrimination would be more difficult to detect. The long-term experience experiment provided direct evidence that such an effect did not occur with cabbage, as experience on rape neither increased nor decreased acceptance of cabbage relative to cabbage experienced moths. With the present data however, I cannot completely rule out the possibility that experience on rape increased acceptance of cress by deprived moths, or decreased acceptance by non-deprived moths – both of which would obscure a decrease in discrimination with deprivation. The latter seems more likely because of the more recent connection between the experience and the test situation. Counting against this interpretation is the strikingly clear importance of very recent experience as demonstrated in the two learning experiments. At this point, without conducting a further experiment, it is not possible to be absolutely certain that rape had no effect on acceptance on cress.

### The Incremental Acceptance Model.

Notwithstanding the final comment made in the preceding paragraph, the data appear to contradict the expectations of the motivation state models. The failure of motivation state models to explain these results leaves a theoretical vacuum in which to interpret observations of herbivorous insect oviposition behaviour. In an attempt to stimulate further research, I tentatively propose the Incremental Acceptance Model. This model combines sensitisation with a threshold mechanism to constitute a variable scheme for ranking the acceptance of

hosts in time according to both host suitability for larval development, and their relative probabilities of being encountered. I have focused on sensitisation to the whole cue profile as the mechanism by which experience is translated into changes in behaviour, although currently there are not enough data to exclude habituation to deterrents as the cause of changes in responsiveness. In the context of this model, either mechanism will function similarly. In this model, I propose that for a host-plant to be accepted, a sensitised internal excitation state to the specific cues of that plant type must exceed an internal fixed threshold value. Chemical and/or morphological similarity rather than taxonomic affiliation determine the plant type. The amount of exposure to a host that is required for the insect's excitation state to exceed the threshold determines how rapidly it will become responsive to the host (Figure 2.1). When responsive to a host, an insect will not reject it on the basis of host-specific cues. Discrimination between host species or types is determined by the relative rates at which the insect becomes responsive to the hosts. Insects perceive the attractiveness of plants by a balance of stimulant and deterrent cues (Renwick & Chew 1994). I suggest that a less attractive plant will require many encounters over a short period before the insect is responsive to it, whereas a highly attractive plant may require only one encounter. Less time will thus elapse before the insect is responsive to a high intensity stimulus than to a low intensity stimulus for the same encounter rate. This is consistent with Singer's findings that acceptance of different quality hosts can be separated in time (Singer 1982). Non-stimulatory plants, which have an excess of deterrents over stimulants, will never be acceptable and may produce aversion. Once responsive to a host, a female will continue to be responsive until sufficient time without stimulation results in relaxation of the excitation state below the response threshold. A response threshold, while difficult to test for in diamondback moth, appears to be consistent with the observations. The existence of a threshold does however, require more widespread testing in addition to that carried out by Singer (1982, 1983). The model focuses on sensitisation as the primary learning mechanism by which responsiveness occurs, but the role of associative learning is less clear. I speculate that associative learning may reinforce behaviours toward an already acceptable host but further work is required to clarify this point.



**Figure 2.1.** *The stages of the Incremental Acceptance Model. Cues required to specifically identify a host species are assessed to determine responsiveness. The process involves sensitisation to the host-specific cue profile of the host species through repeated host exposure. When the sensitised excitation level exceeds some threshold value the insect is 'responsive' to the host. The stimulus intensity and the recent encounter rate with the host species both contribute to the time at which an insect becomes responsive to the host species. In the particular case considered in the diagram, the insect is responsive to species 'A' the first time 'A' is encountered. Ranking is determined by the order in which the insect becomes responsive to host types following the commencement of searching. Oviposition acceptance of a particular host-plant occurs only if the insect is responsive to the host species and, on balance, is stimulated by the non-specific cues. If the insect is either unresponsive or deterred by the non-specific cues then the host-plant is rejected. Non-specific cues may emanate directly from the plant, the local environment, or reflect the internal physiological condition of the insect.*

The Incremental Acceptance Model predicts that any factors that change the rate of encounter with a specific host-plant type will affect the rate at which the insects become responsive to



it. Encounter rates may be modified through a general increase in encounter rates such as when egg-load increases search intensity (Minkenberg *et al.* 1992), a change in plant density (Rausher 1983a), variations in host frequency, or modifications to orientation behaviour through experience (Stanton 1984). I note at this point that there is no reason to suppose that learning in oviposition acceptance decisions implies the presence of learning in all facets of the host finding process. Insect host searching strategies vary considerably, from strategies that encounter potential hosts at random (Parmesan *et al.* 1995), through to those that show a strongly non-random search pattern (Rausher & Odendaal 1987). Learned orientation behaviour is probably associative, linking the cues detected pre-host contact with the response to those encountered post-contact (Landolt & Molina 1996).

The retention of distant experience in a sensitisation process is inhibited by the spontaneous relaxation of sensitised responses (Petrinovich 1984). This was quite clearly evidenced by the absence of any effect of long-term experience on acceptance. I suggest that if a host is sufficiently rare, it is possible that the rate of responsiveness induction may be less than the rate at which the insect forgets previous experience. As a direct corollary, I hypothesise that there exists a minimum frequency of encounter below which a host will never be accepted. For very stimulatory hosts this will not apply as the base level of response to a single presentation may be sufficient to exceed the excitation threshold required for oviposition. The possibility of a minimum frequency of encounter is increased if the retention of old host information is constrained by the memory capacity of the insect. Inhibition of recall of distant experience by more recent experience has been found in the pipevine swallowtail *Battus philenor* (Rausher & Odendaal 1987). Switching from the current search image by *Battus* is dependent on ‘mistakenly’ alighting on the species of *Aristolochia* not currently searched for. In the parasitic wasp *Leptopilina boulardi*, the last odour learnt is preferred although it also has memory for at least two host-associated odours (De-Jong & Kaiser 1992). A minimum encounter frequency for acceptance may, in addition to the apparency of rare plants, account for the tendency for rare plants to escape herbivory (see Chapter 3 for examples).

As might be expected, if an insect is responsive to a specific host type, this won’t always result in the laying of an egg on a particular individual plant of that type. By way of the Incremental Acceptance Model, I suggest that a female insect will only oviposit if she is both responsive and finds the plant suitable in a non-specific way (Figure 2.1). The response to the general cue, deprivation, was not dependent on host-specific responsiveness in the present study, suggesting that the evaluation of host-specific risk is separate from the assessment of

general risk. I define a general cue as one whose presence or absence does not correlate with the presence of a specific host phenotype. Instead, these cues indicate the level of non-specific risk or suitability for larval survival of choosing to accept a particular plant. These general cues could regulate the oviposition reflex in response to a specifically identified host type or species. Further validation that specific and non-specific cues affect acceptance independently of each other is still required. If general cues regulate the oviposition reflex, they could determine the distribution of eggs between and within plant types, and in doing so, account for the observed increases in acceptance rates with egg-load. For example, *B. philenor* became more discriminating within a host species as the rate of oviposition increased (Rausher 1983a). In a somewhat complicated argument, Rausher rejected the possibility of egg-depletion as the cause for this observation. He argued that because the alighting rate on plants was linearly related to density, the proportion of plants that were laid on should have been constant with increasing density until the point at which the rate of egg-depletion exceeded the rate of egg-maturation. At this point there should have been a steady decline, rather than from the very lowest densities that were present as observed in the data. Crucially however, he failed to demonstrate that the rate of egg depletion was less than the rate of egg maturation at the lowest egg-laying rates he observed. Examples of external and internal non-specific cues that can influence oviposition include odours associated with competitors (Dempster 1992; Messina *et al.* 1992), female age (Stanek *et al.* 1987), humidity (Pivnick *et al.* 1990), general plant features such as surface texture (Kumar 1992), and host deprivation as demonstrated in the present study. While it is possible that evaluation of these cues might also involve some form of learned response, there is currently no evidence to support this. Instead as a null hypothesis, I suggest that insects evaluate general cues as a balance between stimulatory and inhibitory cues on a host by host basis. Some evidence for this assertion comes from the seed beetle *Callosobruchus maculatus*. *Callosobruchus* discriminates between seeds with conspecific eggs, and those without, producing a nearly uniform distribution of eggs between seeds (Messina *et al.* 1992). However, when deprived of seeds for a period of ten hours, two and a half times as many beetles laid eggs on seeds already with eggs present than the non-deprived group, suggesting a direct balance between the stimulant cue (host deprivation) and the deterrent cue (conspecific eggs).

The generality of the behavioural mechanism hypothesised here would be questionable if a number of studies have found no evidence for learning in host acceptance. Thomas and Singer (1987) claimed to find no evidence that learning affects acceptance in *Euphydryas editha*. Their trial involved training butterflies with nine sequential presentations of either

*Pedicularis semibarbata* or *Collinsia torreyi* followed by a no-choice test on either host-plant. Butterflies were collected from an area in which butterflies show a strong preference for *Pedicularis* over *Collinsia* (Singer 1983; Thomas & Singer 1987) yet they did not discriminate between either host regardless of the plant used in training. This strongly suggests some sort of unidentified experimental effect. Unfortunately Thomas and Singer did not have an untrained control, so I can only speculate on the possible causes, but these might include temporal conditioning or perhaps cross induction of preference. Scriber (1993) also found no evidence of induction of preference in a four-way choice test. Scriber counted eggs every 24 hours and as he noted, it is quite possible that an effect of training occurred early on in the test period that was masked by subsequent experience gained during the test procedure. Given that learning can occur with only a few seconds contact with a host (Landolt & Molina 1996) and has been shown to occur in the present study with 15 minutes of plant exposure, 24 hours is a long time to leave insects in a test situation.

### Concluding remarks

The data presented in this chapter seriously challenge the pre-eminence of motivation state models as an explanation for herbivorous insect oviposition behaviour. In proposing the Incremental Acceptance Model, I wish to offer a new perspective on oviposition acceptance behaviour. The model makes a significant departure from earlier models of host acceptance by developing new ideas on the relationship between host-specific and non-specific cues in determining host-plant acceptance. It also centralises the role of learning in determining the ranking of host acceptance in time while the expression of this preference is controlled by non-specific cues. Such an approach makes intuitive sense in that it allows the insect to respond to broad host variation at the patch level while at the same time exercising some control over choice between individual plants. By offering an alternative interpretation on the role in host acceptance behaviour of the deprivation associated effects of egg-load and time since last oviposition, the incremental acceptance model suggests that a more rigorous approach than taken in the past is required to test these relationships.

## CHAPTER 3

# THE EFFECT OF THE PLANT INDUCTION STATE ON THE INTRA-SPECIFIC PREFERENCE OF THE DIAMONDBACK MOTH

### *INTRODUCTION*

Adapted insects, against which a plant's morphological and chemical defences are ineffective, cause significant injury to plants (Mopper *et al.* 1990; Quiring & Butterworth 1994). Many of these insects are specifically attracted to the defences to which they are adapted (Stadler & Buser 1984; Tallamy 1985; Metcalf & Lampman 1989). One way that plants can prevent adapted herbivory is through avoidance by using an escape strategy (Feeny 1975, 1976). There is a diverse array of escape mechanisms. Some escape mechanisms depend on a lack of synchrony between plant development and the insect population's ability to respond. Examples of this type of mechanism include satiating predators by producing numerous offspring (Donaldson 1993), cryptic timing of developmental processes (Tuomi *et al.* 1989; Euler & Baldwin 1996; Lawrence *et al.* 1997) and having short generation times as suggested by Feeny (1975, 1976). Another possible strategy involves escape by spatial association with other plant phenotypes (Henderson 1990; Karban 1997) by taking advantage of weaknesses in the insect's host-detection mechanism. In this case the associated plant may mask the presence of the escapee by deterring the insects (Till-Bottraud & Grouyon 1992) or by being preferentially utilised (Hunt & Whitfield 1996). Escape by association depends on the escapee being sufficiently rare as to be cryptic (Srinivasan *et al.* 1994). Ironically by being too successful, and hence common, the value of the mechanism is decreased.

Escape by association may also occur within populations due to intra-specific variation in plant phenotypes, and may be an important factor in the distribution of herbivory within a plant population, particularly when this variation is induced by herbivore feeding. Herbivore induced variation offers the intriguing prospect of feedback loops in which the choices of previous insects affect the decisions made by subsequent herbivores. Examples of herbivore induced plant responses are numerous (reviewed Karban & Myers 1989; Karban & Baldwin 1997), but the outcome for plant escape depends on the nature of the response. If insects are deterred by the particular induced change, the result can be a more homogeneous distribution of insects (Wold & Marquis 1997) because newcomers preferentially settle on unoccupied

plants. However, if insects are preferentially attracted to the induced phenotype, such as occurs with many adapted insects (Lindstrom *et al.* 1990; Tallamy & McCloud 1991; Landolt 1993; Uematsu & Sakanoshita 1993; Pivnick *et al.* 1994; Baur *et al.* 1996; Anderson & Alborn 1999), then insect distributions may become over-dispersed allowing uninduced plants to escape. As in inter-specific escape, we might expect that phenotype frequency has an important role, however the effects of changes in the frequency of induced phenotypes on predation by adapted herbivores has received little attention. An accurate assessment of the fitness consequences for plants of herbivore induced responses requires knowledge on how the observed phenotype frequencies moderate the risk of herbivory. This assessment also requires an understanding of the factors affecting insect host choice between phenotypes

A herbivore's response to induced phenotypic variation is probably a very plastic characteristic of an insect's behaviour. Chapter 2 highlighted how oviposition responsiveness may change with adult experience, but it has also been suggested that it may change with juvenile experience. Hopkins proposed that larval experience gained on a particular host would increase the adult's preference for that host (Hopkins 1917). According to this argument, information regarding host suitability – as evidenced by the survival of the individual through to adulthood – is translated into an increase in the probability of oviposition on similar hosts either through learning, or a modification of the insect's sensory system. Evidence for the 'Hopkins host selection principle' has been difficult to obtain and some authors believe the principle to already be discredited (*e.g.* van Emden *et al.* 1996). Difficulty has arisen because most studies claiming to support the hypothesis have failed to isolate larval-experience-derived changes in behaviour from either the effects of genetic specialisation on particular hosts, chemical residues left on cocoons or early adult contact with host plants (Corbet 1985; Szentesi & Jermy 1990). Solarz and Newman (1996) for example, have claimed that the larval host affected oviposition preference in the weevil *Euhrychiopsis lecontei*, but as the insects were not removed from the host plants prior to adult emergence, neither chemical residue or early adult experience can be ruled out. In addition to these methodological difficulties, further doubt is cast upon the Hopkins principle by a number of studies that have found no effect of larval experience on adult preference (*e.g.* Rausher 1983b; Firempong & Zalucki 1991; Lee & Tseng 1993; van Emden *et al.* 1996; Rojas & Wyatt 1999).

Traditionally, the test for Hopkins host selection principle involves preference trials on intact plants using adults reared as larvae on different hosts. This design pre-supposes that intact

plants represent a similar stimulus to that experienced in the larval environment. The validity of this assumption is questionable given the widespread occurrence of feeding induced changes in plant quality. The use of alternative test stimuli such as larval frass for *Spodoptera littoralis* (Anderson *et al.* 1995) has already been shown to yield evidence in favour of the Hopkins principle. Pre-damaged host plants could also be used as more relevant test stimuli given that larvae experience damaged plant environments when feeding. It is important to quantify the role of larval experience in host choice as most preference tests use naive insects, which may lead to erroneous conclusions on patterns of host use if adult experience takes precedence in determining oviposition preference.

This chapter examines the effect of damage induced changes to the phenotypes of *Brassica spp.* on the oviposition behaviour of diamondback moth (*Plutella xylostella* L. (Lepidoptera: Yponomeutridae)). Mustard oils are produced in several *Brassica* species in response to mechanical and insect damage to leaf tissues (Pivnick *et al.* 1994). Using the terminology of Karban and Baldwin (1997), mustard oils may be viewed as “pre-formed induced chemical responses”, although over longer periods glucosinolates (the chemical precursors to mustard oils) can increase in response to herbivory (Bodnaryk 1992, 1994). Mustard oils act as deterrents to many herbivores (Nault & Steyer 1972) but are highly stimulatory to diamondback moths (Pivnick *et al.* 1994). In addition, diamondback moth has been shown to prefer previously damaged plants (Uematsu & Sakanoshita 1993). This makes this moth highly suited to study how the distribution of eggs varies with the relative frequency of the damaged and undamaged phenotypes. The Hopkins host selection principle is also tested by looking at the role of the larval host plant in determining the relative acceptance probabilities of damaged and undamaged plants. Lee and Tseng (1993) found no effect of rearing host on diamondback moth preference for intact plants and so this provides a useful opportunity to also test the appropriateness of damaged plants as a test stimulus.

## METHODS

In all experiments, cabbage refers to *Brassica oleracea* cv. “Golden Acre Merit” and rape refers to *Brassica napus* cv. “Rangi”. These plant species were chosen as previous work has shown that damaged induced changes in the plant phenotype result in changes in host plant preference for female diamondback moths (Uematsu & Sakanoshita 1993; Pivnick *et al.* 1994). All plants were grown in fertilised potting mix in the University of Canterbury glasshouses. Natural light was supplemented by 400W sodium lighting to give a 16 hour day

length. All testing was carried out in complete darkness to minimise visual stimuli, at a temperature of  $21 \pm 2$  °C.

### Two-way choice test

Moths were reared on 25 day old cabbage plants. Upon emergence of adults, males and females at a 2:1 sex ratio were allowed to mate in 20 cm high, 4 litre plastic cages, which had mesh on the top and one side. This sex bias in favour of males was used because only 54% of males are sexually mature by the first night following emergence (Pivnick *et al.* 1990a). The following night, each two day old female was transferred to a 0.5 x 0.5 x 0.5 m cage enclosed in black plastic except for mesh on the roof and one side. At the beginning of the scotophase, two 16 day old cabbage seedlings (one mechanically damaged, one undamaged) were placed in each cage in a two-way choice test. The mechanically damaged plants were damaged using a device with a bed of 2 mm nails to punch holes in the leaf surface. Approximately 3% of the total leaf area per plant was holed. Eggs were counted after a 30 minute test period.

### Patch composition – experiment I

Moths were reared on rape. One female and three male moths were transferred to a 0.5 x 0.5 x 0.5 m cage at emergence. They were given access to a single intact rape plant and a 10% sucrose solution cotton wick feeder. Once females had laid a minimum of 20 eggs during a single night they were judged to be sufficiently sexually mature as to be ready for testing. This is somewhat less than the 60 eggs used by Reed *et al.* (1989), however in general the fecundity of my moths appeared to be fairly low. Using such a criterion had the unfortunate disadvantage that moths varied in age by up to three days when tested. Age has been shown to affect acceptance behaviour in diamondback moth (Pivnick *et al.* 1990b) and other insects (Papaj & Rausher 1983; Stanek *et al.* 1987), but it is difficult to ascertain whether this was a significant source of variation.

The experimental regime followed a cross-over design in which the experimental unit is an individual subject during some short period of time (Mead 1988, p197). Each subject is given every treatment and each treatment should be preceded by every other treatment exactly once for each complete replicate. The design is essentially a replicated Latin square with period and subject as blocks, and as many periods and subjects per replicate as treatments. The design allows the experimenter to examine the effects of treatments applied sequentially to the subject (Mead 1988), much like an insect encounters multiple patches in sequence during

its oviposition lifetime. Sufficient replication is only feasible to examine the prior effects at one time period removed from current, however the effects of experience reported in Chapter 2 suggest that experience gained more than one day before testing is unlikely to be important. This design was chosen in anticipation of a high level of variability between subjects, such as has been reported for other Lepidoptera (*e.g.* Wiklund 1981; Singer 1982, 1983). Using individuals as blocks improves the precision with which experimental effects can be estimated (Mead 1988).

Treatments consisted of patches containing nine potted 18 day old rape plants arranged in a three by three grid. Four combinations of damaged and undamaged plants were used:

- All undamaged (all nine plants undamaged)
- Damaged-rare (seven undamaged, two damaged)
- Undamaged-rare (two undamaged, seven damaged)
- All damaged (all nine plants damaged)

Damaged plants had 5 mm holes punched in the leaves using a cork-hole borer 10 minutes prior to testing. Approximately 15% of each leaf was removed, corresponding to the level of damage at which Riggin-Bucci and Gould (1996) claimed to have found an effect on diamondback moth oviposition.

The damaged and undamaged plants were arranged randomly within the three by three grid. The patches were placed in the cage at the beginning of the scotophase and removed eight hours later when the eggs on each plant were counted. The moths were given one patch per day. Three separate runs were carried out, with each run consisting of two complete replicates.

Egg counts were analysed with Poisson generalised linear models (GLM's) with log link functions. The analysis of egg-laying probabilities on rare plants used binomial GLM's with logit link functions. The significance of the effects were assessed using two tailed  $\chi^2$ . *F* tests were used when the data were over-dispersed (Crawley 1993). All GLM's were fitted using S-Plus version 4.5 (Mathsoft Inc. 1998). One sample t-tests on arcsin transformed data were used to test whether the proportions of eggs found on each host plant phenotype differed from the random expectation. Minimum detectable differences and probabilities of Type II errors of the one sample t-tests were calculated as per the methods of Zar (1984, p111). The



significance of the comparisons of relative herbivore loads per plant were assessed by ANOVA on inverse transformed data.

### Patch composition – experiment II

The complexity of the cross-over design, and a general unwillingness of moths to lay eggs, necessitated a change to a more simple design to test the effect of patch composition on the distribution of eggs within the patch. This experiment focused on the two patches containing both damaged and undamaged plants and did not include any form of prior experience.

Moths were reared and tested on cabbage. A switch to cabbage was made as cabbage had been extensively used in the trials conducted in Chapter 2. Male and female moths at a sex ratio of 2:1 were placed in a holding cage following hatching and allowed to mate freely during the first night. Individual females were transferred to the 0.5 x 0.5 x 0.5 m test enclosures four hours prior to testing for acclimatisation. At the beginning of the scotophase, females were given either a damaged-rare patch or a undamaged-rare patch as described previously. Plants were damaged 10 minutes prior to testing using the nail punch machine. A change to the nail punch machine was made because it produces more uniform damage (3% of leaf area) and is considerably faster. The test plants were removed four hours later and the eggs counted. The test period was shorter than used previously to minimise the possibility that the surface chemicals released following damage had evaporated completely during testing. Apparently diamondback moths lay most of their eggs during this first four hour period of the scotophase (Talekar & Shelton 1993). Females were tested once and then discarded.

The analysis was as for the previous patch composition experiment except that the GLM's modelled day as a block and patch composition as the sole factor. In addition, relative herbivore loads were analysed using Wilcoxon rank-sum tests (S-Plus, version 3.3, Mathsoft Inc. 1995).

### Larval experience

Approximately 150 moths from a laboratory culture raised on rape for at least 15 generations were placed in an egg-laying cage and allowed to oviposit on 10 day old rape seedlings. Sourcing the moths from a common genetic line ensured that differences in the behavioural responses of the insects to different rearing regimes were not due to genetic differences, as

could be the case if both treatment groups were reared on their respective training plant species for greater than one generation (Szentesi & Jermy 1990). Upon hatching, first instar larvae were transferred to 6 week old rape or cabbage at a density of six larvae per plant. An effect of this very early experience on rape seedlings ought to be negligible, as an effect of larval experience on larval preference is not detectable until the fourth instar (Lee & Tseng 1993). Following pupation, pupae were removed from their cocoons and allowed to emerge on filter paper in clean petri dishes to reduce the chance of contamination by host plant material (van Emden *et al.* 1996). A sample of pupae from each rearing treatment was weighed.

Following emergence, male and female moths at a sex ratio of 2:1 were placed in a holding cage and allowed to mate freely during the first night. The following night, each 2 day old female was transferred to individual 4 litre test cages one hour before testing. The 4 litre cages are small enough that the influence of orientation behaviour on acceptance would have been minimal, hence the test was effectively one of post-alighting preference. At the beginning of the scotophase, each moth was given either an intact or an artificially damaged 18 day old cabbage plantlet for 45 minutes in a no-choice test. Damaged plants had holes punched in the leaves using the nail punch machine. Eggs on the test plants were counted. An insect was recorded as having accepted the host if at least one egg was laid on the plant during the test period. The data were analysed using two-tailed  $\chi^2$  tests.

## **RESULTS**

### **Two-way choice test**

Eggs were laid in only 13 of the 24 replicates in this experiment. The analysis is confined to replicates in which eggs were laid. Significantly more eggs were laid on damaged plants than on undamaged plants (mean  $\pm$  SE =  $11.2 \pm 4.5$  and  $1.8 \pm 0.8$  respectively; paired t-test,  $t_{12} = 2.05$ ,  $p = 0.032$ ).

### **Patch composition – experiment I**

#### **Whole patch**

43 of the 96 patches (44.8%) did not receive any eggs (Table 3.1). The presence or absence of eggs was significantly affected by the damaged/undamaged composition of the patch

**Table 3.1.** *The percentage of patches that were laid on during the first patch composition experiment. The sample sizes are shown in brackets. Previous experience on the small cage is synonymous with patches tested on day one.*

Patch	% of patches with eggs	
	Patch = current day's patch	Patch = previous day's patch
All undamaged	41.7 (24)	61.1 (18)
Damaged rare	45.8 (24)	66.7 (18)
Undamaged-rare	70.8 (24)	38.9 (18)
All damaged	62.5 (24)	61.1 (18)
Small cage (day 1)	NA	50.0 (24)

(binomial GLM with logit link,  $\chi^2_3 = 14.29$ ,  $p = 0.003$ ) and by patch-dependent prior experience ( $\chi^2_3 = 8.62$ ,  $p = 0.038$ ). Those patches with the highest response probability also had the highest proportion of damaged plants. In addition it appeared that the moths laid fewer eggs when the previous day's patch was undamaged rare.

There was no effect of patch composition (Poisson GLM with log link,  $F_{3,52} = 0.25$ ,  $p = 0.86$ ) or the previous patch experienced ( $F_{3,14} = 0.64$ ,  $p = 0.60$ ) on the number of eggs laid in a patch given that an egg was laid (Table 3.2). The composition by prior experience interaction was also non-significant ( $F_{8,14} = 0.83$ ,  $p = 0.59$ ). If an effect of prior experience was a function of learning then it may be more accurately represented by the similarity between the previous and the current patch. However in common with the previous analysis, replacing prior experience with a simple index of similarity also does not indicate a significant effect of previous experience (Table 3.2; Poisson GLM, Similarity main effect  $F_{2,13} = 0.43$ ,  $p = 0.66$ ). These data indicate that once a patch is acceptable, insects will continue to lay eggs until the same state of egg depletion regardless of the patch.

### Choice within patches

Confining the analysis to mixed phenotype patches in which eggs were laid, we can ask whether previous experience and the composition of the patch affected the distribution of eggs between plant phenotypes within a patch. Unfortunately there were not enough data to

**Table 3.2.** *The average number of eggs laid per time period given that an egg was laid in the first patch composition experiment, grouped by patch composition, prior experience and similarity. Similarity is defined as the proportion of plant phenotypes in the previous patch that are shared by plants in the current patch (i.e. When the number of individuals of each phenotypes is given by A and B respectively, Similarity =  $(\min(A_{\text{current}} - A_{\text{previous}}) + \min(B_{\text{current}} - B_{\text{previous}}))/9$ ). Data from the first time period are excluded from the similarity results as these do not have a comparable previous patch. Data are given as the mean  $\pm$  SE. Sample sizes are given in brackets.*

Patch	Mean eggs		Similarity	Mean eggs
	Patch = current day's patch	Patch = previous day's patch		
All undamaged	32.9 $\pm$ 5.1 (10)	28.8 $\pm$ 6.2 (11)	0	32.6 $\pm$ 6.6 (9)
Damaged-rare	22.2 $\pm$ 4.3 (11)	35.1 $\pm$ 5.7 (12)	0.222	26.5 $\pm$ 4.5 (13)
Undamaged-rare	28.7 $\pm$ 5.8 (17)	23.7 $\pm$ 6.5 (7)	0.444	32.3 $\pm$ 7.6 (8)
All damaged	29.1 $\pm$ 5.1 (15)	29.3 $\pm$ 7.2 (11)	0.778	30.1 $\pm$ 7.9 (11)
Small cage (day 1)	NA	22.6 $\pm$ 4.3 (12)		

test for more than one factor at a time ( $N = 28$ ) due to the high number of patches that were not laid on. The integrity of the experiment has been compromised by the inability to model the effects of current patch and previous patch simultaneously because these factors are partially confounded. Prior experience on either of the mixed patches is confounded with the current composition of the alternative mixed patch as no insect received the same patch twice (i.e. prior experience on damaged-rare patches were always tested on undamaged rare patches for this subset of the data and vice-versa). This presents a problem of interpretation and the limitations of the analysis should be recognised.

There was a significantly higher probability of laying on a rare phenotype when it was damaged than when it was not (Table 3.3; binomial GLM,  $\chi^2_1 = 5.55$ ,  $p = 0.019$ ). The data presented in Table 3.3 indicate that previous experience on undamaged rare patches may inflate the probability of laying on rare phenotypes in the damaged rare patch. While no effect of previous patch was detected (binomial GLM,  $F_{3,3} = 1.39$ ,  $p = 0.40$ ), this conclusion is weak because of the inability to test for interactions. Nevertheless, for all types of previous experience, acceptance of the rare phenotype was higher when the current patch was

**Table 3.3.** *The probability that the rare phenotype is laid on for each mixed patch sorted by previous patch (mean  $\pm$  SE). The sample sizes are given in brackets.*

Current Patch	Previous patch					Overall
	All damaged	Undamaged rare	Damaged rare	All undamaged	Small cage (day 1)	
Damaged-rare	0.333 $\pm$ 0.333 (3)	1 $\pm$ 0 (3)	NA	0.500 $\pm$ 0.500 (2)	0.333 $\pm$ 0.333 (3)	0.545 $\pm$ 0.157 (11)
Undamaged-rare	0.250 $\pm$ 0.250 (4)	NA	0.400 $\pm$ 0.245 (5)	0.250 $\pm$ 0.250 (4)	0 $\pm$ 0 (4)	0.235 $\pm$ 0.106 (17)

damaged-rare, indicating that the significant effect of current patch is probably robust, even allowing for the effects of prior experience on undamaged rare patches. Given that a rare phenotype was laid on, a greater proportion of eggs also appeared to be laid on rare damaged plants than rare undamaged plants (mean = 47.3% and 22.8% respectively), however there were too few data to test this. Overall, despite rare damaged plants being more likely to be laid on than rare undamaged plants, no effect of patch composition (Table 3.4; binomial GLM,  $F_{1,5} = 0.163$ ,  $p = 0.703$ ), or previous experience ( $\chi^2_3 = 0.12$ ,  $p = 0.99$ ), on the proportion of eggs laid on the rare phenotype was detected.

A significantly greater proportion of eggs were laid on each phenotype when common than

**Table 3.4.** *The proportion of eggs laid and the relative herbivore load on each phenotype in mixed patches only, during the first patch composition experiment. Relative herbivore load = proportion of eggs/phenotype frequency.*

Patch	Mean proportion of eggs on each phenotype		Mean relative herbivore load	
	Damaged	Undamaged	Damaged	Undamaged
Damaged-rare	0.258	0.742	1.16	0.95
Undamaged-rare	0.946	0.054	1.22	0.24
Common vs. rare (comparison within columns)	binomial GLM, $F_{1,5} = 25.44$ , $p = 0.004$		ANOVA, inverse transform, $F_{1,5} = 1.72$ , $p = 0.25$	ANOVA, inverse transform, $F_{1,5} = 2.98$ , $p = 0.14$

when rare (Table 3.4), indicating a positive relationship between phenotype frequency and the probability of receiving eggs. However, a departure from the random expectation for the distribution between phenotypes was only detected in undamaged rare patches (undamaged-rare, one sample t-test,  $t_{16} = 6.95$ ,  $p \ll 0.0001$ ; damaged-rare,  $t_{10} = 0.388$ ,  $p = 0.71$ ). Even allowing the low resolving power of the test for damaged-rare patches (minimum detectable difference = 0.19, for power = 0.8,  $\alpha = 0.05$ ), this conclusion would seem to be robust based on the observed and expected means (0.258 and 0.222 respectively). We can also ask whether the higher probability of receiving eggs when common translates into a greater risk per plant by scaling the proportion of eggs by the relative frequency of the plant phenotype. No difference between patches in the relative herbivore load per plant was detected (Table 3.4).

Ignoring differences between phenotypes, there was no effect of patch composition on the distribution of eggs between individual plants within patches. The frequency distributions of the number of plants with eggs were similar for homogeneous and mixed patches ( $\chi^2_6 = 0.53$ ,  $p = 0.997$ ). Therefore despite rare plants being more likely to be laid on if damaged than undamaged, individual moths don't appear to concentrate their eggs on fewer hosts when in mixed versus homogeneous patches. This is probably because the majority of ovipositing moths appeared to lay on only none or two plants ( $56.6\% \leq 2$  plants).

## Patch composition - experiment II

### Whole patch

Of the 40 patches, 24 did not receive any eggs. There was no systematic effect of patch composition on acceptance of a patch (binomial GLM,  $\chi^2_1 = 0.47$ ,  $p = 0.49$ ). As such, patches in which no eggs were laid contributed no useful information on insect responses, and were excluded from the subsequent analyses.

**Table 3.5.** *The distribution of eggs in damaged-rare and undamaged-rare patches for the second patch composition experiment.*

Patch composition	<i>N</i>	Total eggs (mean $\pm$ SE)	Probability that rare phenotype was laid on	Mean % eggs on rare phenotype
Damaged-rare	9	13.4 $\pm$ 3.5	0.556	53.8 <sup>Ψ</sup>
Undamaged-rare	7	20.7 $\pm$ 5.5	0	0

Ψ The percentage of eggs laid on rare damaged plants given that the rare phenotype was laid on is 96.9%.

There was no significant difference in the total number of eggs laid on either patch (Table 3.5; Poisson GLM,  $F_{1,10} = 0.789$ ,  $p = 0.40$ ). In contrast to the first patch composition experiment, this result, coupled with the independence of patch acceptance from patch type, indicate that the whole patch had no effect on oviposition.

### Choice within patches

No eggs were laid on undamaged plants when rare, and they were significantly less likely to be laid on than rare damaged plants (Table 3.5; binomial GLM,  $\chi^2_1 = 6.05$ ,  $p = 0.014$ ). Consequently, a greater proportion of eggs were also laid on rare plants when they were damaged (binomial GLM,  $\chi^2_1 = 5.64$ ,  $p = 0.018$ ).

Each phenotype was more likely to be laid on when common than when rare (Table 3.6). While there was a strong trend in both patches for the distribution of eggs between phenotypes to depart from the accepted frequency, this was only significant for undamaged rare patches (undamaged-rare, one sample t-test,  $t_6 = 2.92 \times 10^{15}$ ,  $p < 0.0001$ ; damaged-rare,  $t_8 = 1.27$ ,  $p = 0.24$ ). The non-significant result for damaged rare patches must be treated with caution however, as the probability of a Type II error was high ( $\beta = 0.754$ ). Undamaged plants had a significantly higher relative herbivore load when common than when rare (Table 3.6). No difference in herbivore load with rarity was detected for damaged plants.

**Table 3.6.** *The proportion of eggs laid, and the relative herbivore load, on each phenotype for mixed patches in the second patch composition experiment. Relative herbivore load = mean proportion of eggs/phenotype frequency.*

Patch	Mean proportion of eggs on each phenotype		Mean relative herbivore load	
	Damaged	Undamaged	Damaged	Undamaged
Damaged rare	0.538	0.462	2.42	0.59
Undamaged rare	1	0	1.29	0
Common vs. rare (Comparison within columns)	binomial GLM, $\chi^2_1 = 6.50$ , $p = 0.011$		Wilcoxon rank-sum, $Z_{7,9} = 0.335$ , $p = 0.738$	Wilcoxon rank-sum, $Z_{7,9} = 2.15$ , $p = 0.032$

Again there was no effect of patch composition on the frequency distribution of the number of plants with eggs ( $\chi^2_3 = 1.48$ ,  $p = 0.69$ ). Only one insect laid eggs on more than two plants.

### Larval experience

The pupae of females reared on cabbage were slightly heavier than those reared on rape (8.7 and 8.4 mg respectively; two-tailed t-test,  $t_{62} = 2.13$ ,  $p = 0.04$ ). There was no significant effect however, of the plants used for rearing on the number of eggs laid by those females that accepted hosts (mean  $\pm$  SE: cabbage =  $11.8 \pm 2.1$ ; rape =  $9.3 \pm 1.5$ ; Poisson GLM,  $F_{1,51} = 0.054$ ,  $p = 0.82$ ). Therefore I consider that there was a minimal effect of insect condition, correlated with rearing plant, on the general motivation to oviposit.

Oviposition acceptance probabilities for moths reared on cabbage or rape, and tested in a no-choice design, are shown in Table 3.7. In contrast to expectations, cabbage reared moths showed no significant preference for damaged over undamaged cabbage ( $\chi^2_1 = 0.172$ ,  $p = 0.68$ ). Surprisingly, moths reared on rape preferred damaged cabbage ( $\chi^2_1 = 5.59$ ,  $p = 0.018$ ). No effect of rearing plant on acceptance of undamaged cabbage plants was detected ( $\chi^2_1 = 0.0059$ ,  $p = 0.94$ ), although rape reared insects were more likely to lay on damaged plants than those reared on cabbage ( $\chi^2_1 = 4.08$ ,  $p = 0.043$ ).



**Table 3.7.** *The effect of larval experience on oviposition acceptance of damaged and undamaged cabbage by diamondback moths. Rows and columns followed by the same letter are significantly different at  $p < 0.05$  (see text for details).*

Rearing plant	Damaged cabbage		Undamaged cabbage	
	<i>N</i>	% accepting host	<i>N</i>	% accepting host
Cabbage	35	34.3 <sub>a</sub>	37	29.7 <sub>a</sub>
Rape	38	57.9 <sub>b</sub>	36	30.6 <sub>a</sub>

### DISCUSSION

A summary of results is given in Table 3.8. Each effect identified in Table 3.8 will be addressed in turn.

**Table 3.8. A summary of the experimental results obtained in Chapter 3.**

Experiment	Effect of		
	Damage	Rarity	Prior experience
Two-way choice	More eggs laid on damaged cabbage plants.		
Patch experiment I	<p>Patches with more damaged plants were more likely to be laid on.</p> <p>Rare phenotypes were more likely to be laid on if damaged.</p> <p>No effect of the rare phenotype on the proportion of eggs that were laid on it.</p>	<p>Plant phenotype significantly less likely to be laid on if rare than when common.</p> <p>Eggs were randomly distributed between the phenotypes in damaged-rare patches, but undamaged plants when rare were less likely to be laid on than random.</p> <p>No effect of rarity on relative herbivore risk.</p>	<p>Less likely to lay eggs on a patch if given undamaged rare the previous day.</p> <p>No detectable effect of adult experience the previous day on:</p> <ul style="list-style-type: none"> <li>• number of eggs laid in a patch</li> <li>• % eggs laid on rare phenotype</li> </ul>
Patch experiment II	<p>Rare phenotypes were more likely to be laid on when damaged.</p> <p>Greater proportions of the eggs were laid on the rare phenotype when damaged.</p>	<p>Plant phenotype significantly less likely to be laid on if rare than when common.</p> <p>Non-random allocation between patches was not detected for damaged-rare patches but undamaged plants when rare were less likely to be laid on than random.</p> <p>Effect of rarity on the relative herbivore risk for undamaged but not damaged plants.</p>	
Larval experience	<p>No effect of the damage state of cabbage on acceptance if reared on cabbage.</p> <p>Strongly prefer damaged cabbage if reared on rape.</p>		<p>No effect of larval experience on acceptance of undamaged cabbage.</p> <p>More likely to accept damaged cabbage if reared on rape rather than cabbage.</p>

## Effect of damage

The experiments in part confirm the findings of Uematsu and Sakanoshita (1993) that diamondback moth preferentially lay their eggs on previously damaged plants when given a choice with intact plants. A preference for damaged plants was illustrated in several ways. The first is that in the two-way choice experiment more eggs were laid on the damaged plants. Secondly, in the first patch composition experiment, those patches with more damaged plants were significantly more likely to be laid on than those with fewer damaged plants. In addition, both patch composition experiments found that the rare phenotype within a patch was much more likely to be accepted if it was damaged compared with undamaged. In the second patch composition experiment this also translated into a greater proportion of eggs on the rare phenotype when it was damaged. In the first experiment the trend was in a similar direction, although it was not significant.

In contrast to the two-phenotype choice tests, the no-choice tests present a less convincing argument for preference for the damaged phenotype. In this experiment, moths reared on rape preferred damaged over undamaged cabbage, but not if they had been reared on cabbage. On the face of it these data seem to contradict the results of the patch composition and two-way choice experiments. Certainly, if the no choice test in the small cages can be taken as a measurement of post-alighting preference, it would appear that damaged preference in the two-way test and the second patch composition experiment could not arise from post-alighting discrimination. Unfortunately these results are less comparable for the first patch experiment, as although the larvae were reared on rape, they were also tested on rape rather than cabbage as used in the no-choice trials. There are also the problems of interpretation with this first patch composition experiment given the partial confounding of previous experience with the current patch. The absence of a preference in the no-choice tests by cabbage reared moths also indicates that the moths did not discriminate between the two phenotypes based on the physical presence of the holes in the damaged leaves. Discrimination based on the holes would require post-alighting discrimination in this nocturnal insect, in which case a preference for damaged plants by cabbage reared moths ought to have also been detected in the no-choice tests.

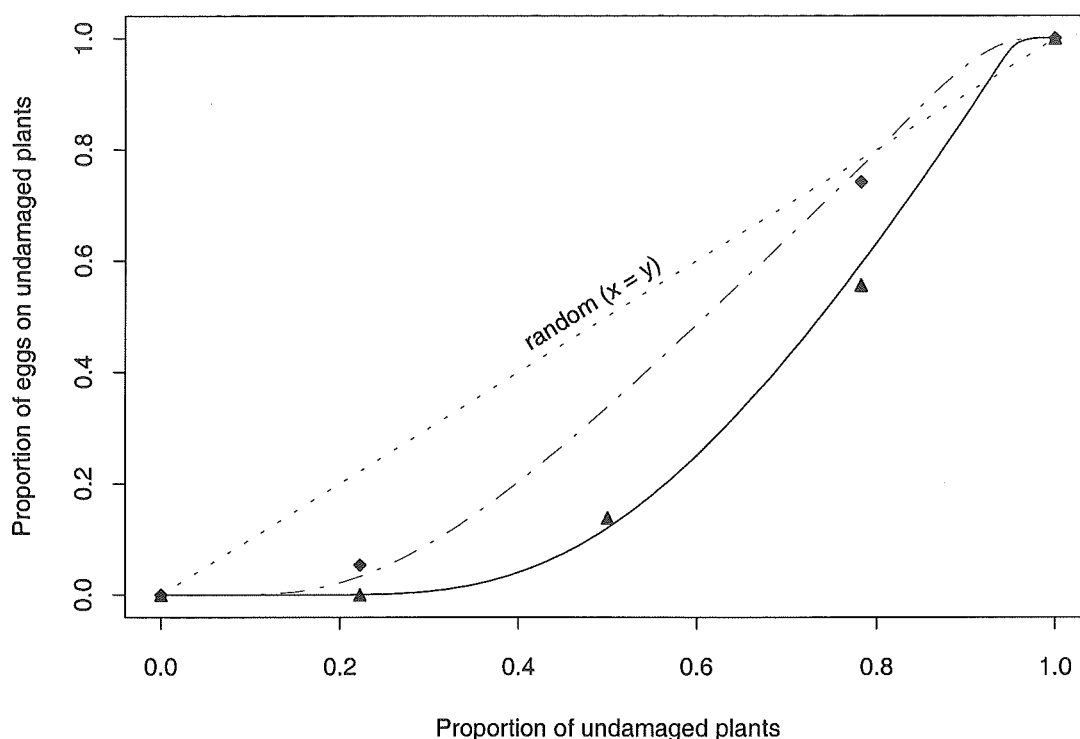
There are several possible explanations for the apparent contradiction between the no-choice and choice tests. It is possible that a change in the quality of damaged plants occurred in the patch composition experiments that did not occur in the no-choice tests because they were tested for a shorter period. Bodnaryk (1992) found that mechanical wounding of cotyledons

in rape produced a detectable increase in the concentration of 3-indolylmethyl glucosinolate in the cotyledons after 4 hours, and in leaves after 7 days. 3-indolylmethyl glucosinolate is an oviposition stimulant to diamondback moth (Reed *et al.* 1989). If direct wounding of the leaves causes a similar but more rapid change in the leaves, then this could potentially explain the preference observed in the first patch experiment for damaged plants. The effect may have been small however, as diamondback moths generally lay most of their eggs during the first 4 hours of the scotophase (Talekar & Shelton 1993). It is a little harder to extrapolate to the second patch composition experiment because these insects were tested on cabbage. Changes in the internal leaf chemistry seem unlikely to explain the difference however, because in the two-way choice experiment (also tested on cabbage), the insects were tested for only 30 minutes - 15 minutes less than the no-choice tests - yet a significant preference for damaged plants was still observed. Another possible explanation is that in the two-phenotype tests there was some form of interaction between phenotypes, in which the presence of undamaged plants enhanced the post-alighting preference for damaged plants. Capinera (1993) for example, found that grasshopper (*Shistocerca americana*) preference for soybean over bahia grass changed according to the other plants present in the system. In a two-way test both hosts were equally preferred. However, three times as many eggs were laid on soybean than bahia grass when other hosts were included in a four-way design. Unfortunately I do not have the data to test this hypothesis, but I consider this to be unlikely in the present study because damaged plants in themselves were also mixtures of damaged and undamaged cues since only 3% of the leaf area was damaged in the larval experience trials. Therefore a synergistic effect between the cues generated by the two plant phenotypes, could also potentially occur with damaged plants only. The final possibility is that the moths discriminated between the phenotypes prior to alighting on hosts in the two-phenotype tests - an opportunity that was not available in the no-choice situation. Pre-alighting and post-alighting preferences are not necessarily correlated (Papaj & Rausher 1983) and for this reason Singer (1986) has called for comparisons between no-choice and multi-choice testing procedures. Pivnick *et al.* (1994) found that diamondback moths preferentially orientated to damaged rape volatiles in a Y-tube olfactometer. They observed that allyl isothiocyanate, the active component released by damaged rape, was virtually absent from intact plant volatiles. Under this scenario, we would expect that when moths are given a choice, they prefer damaged plants because they preferentially locate them during the search process. When given no choice the moths will show no preference such as was observed in the larval experience trials. Based on the evidence, this final explanation seems the most probable.

### Rarity and patterns of host use

In both patch composition experiments, significantly more eggs were laid on plants when common than when rare. However, only in undamaged rare patches was there a significant departure from the expected random distribution of eggs between phenotypes, resulting in undamaged plants being laid on less than expected. While it is possible to argue that in the second patch composition experiment, the test was not powerful enough to detect a difference for the damaged rare patch, this was unlikely to explain the result in the first patch experiment. To invoke random egg-laying with respect to phenotype we also need to argue for a lack of discrimination between phenotypes. Clearly though, at the phenotype frequency used in the undamaged rare patches, the insects did distinguish between damaged and undamaged plants and so it does not seem reasonable that the more favoured damaged phenotype could be cryptic when rare, given that undamaged plants are not. Therefore it seems likely that there could be a systematic effect behind what appears to be a random outcome.

If we accept that this apparently random pattern of egg-laying is the result of chance coincidence between a systematic oviposition response to the phenotype frequency and the value of the frequency itself, then an alternative explanation becomes possible. At a mechanistic level, a moth's contact frequency with a plant phenotype will be dependent on the relative frequency of the host stimulus and the degree of orientation to it. The observed egg distribution will thus be a function of this contact frequency and the encounter-dependent acceptance as hypothesised in Chapter 2. In general, for each plant phenotype in a patch there will be a frequency of occurrence below which they are laid on with probability less than their occurrence frequency. The patch composition experiments demonstrated that this was at least 0.22 for the undamaged phenotype when paired with the damaged phenotype. Conversely, above this relative frequency a plant phenotype will be laid on with a probability greater than that expected at random. This pattern would result in a sigmoidal relationship for the probability of accepting a host for oviposition with host plant frequency in a two-host system because at phenotype relative frequencies of 0 or 1, the acceptance probability is equal to the frequency once again (Figure 3.1). As Figure 3.1 shows, if the preference for damaged over undamaged rape is smaller than the preference for damaged over undamaged cabbage, the oviposition response curve for the first patch composition experiment will have a shorter latency than for the second. Under such a mechanism, the apparently random egg distribution on rare damaged plants could then be the result of a chance coincidence between the value of the sigmoidal response curve for the insect population and the relative frequency



**Figure 3.1.** *Hypothetical sigmoidal response curves fitted to the mean proportion of eggs laid on undamaged plants for both patch composition experiments. The two-way preference test data have been amalgamated with the patch experiment II data as they are identical in experimental set-up except for the number of plants used and the length of the test period. The response curves were fitted by sight using the acceptance model developed in Chapter 4 (p63).*

**Key:** ♦ Experiment I; ▲ Experiment II; alternate dash line: Experiment I fitted curve; solid line: Experiment II fitted curve.

of undefended hosts used in the experiment. Unfortunately it is difficult to discriminate between random behaviour and systematic behaviour that appears random. However a systematic effect could be implied if it could be shown that when damaged plants become even rarer, they are accepted with frequency less than random.

The analysis of the relative herbivore risk for damaged and undamaged plants incorporates the combined effects of the preference for damaged plants and rarity. Only undamaged plants in the second patch composition experiment received significantly lower relative herbivore loads when rare than when common, although a trend in the same direction was observed in

the first patch experiment. By contrast damaged plants were at least as equally likely to have the same relative herbivore load when common as when rare. These data appear to be attributable to undamaged plants escaping herbivory when rare, because eggs in damaged-rare patches were apparently distributed at random between the two plant phenotypes resulting in equal herbivore loads. From the herbivore's perspective, there was no evidence that the eggs were more evenly dispersed between plants in homogeneous patches than in the mixed phenotype patches. With numerous moths all preferentially laying on the damaged phenotype however, we might expect an increase in clumping of herbivores at the population level because plants already damaged by larvae will attract further insects. Hence the cumulative effects of the insect population's laying behaviour could leave some plants with high herbivore loads and others with very little. By concentrating herbivores on a few plants, intra-specific interactions between larvae are increased (Fritz 1990). Life table analysis without time lags indicate that density-dependent effects in diamondback moth in southern Ontario may not be associated with increased death rates or decreased fecundity (Harcourt 1986). By contrast, laboratory trials do show a significant negative effect of larval density on fecundity (Appendix B).

## The role of previous experience

### Adult experience

The evidence that prior experience on a patch affected the adult insect's response to subsequent patches was mixed. There was no effect of previous adult experience on the number of eggs laid in a patch or the proportion of eggs laid on the rare phenotype. These data are consistent with the results of long-term experience obtained in Chapter 2 (Table 2.4) in which no effect of previous host plant experience was also found. However, presentation of the damaged rare patch the previous day in patch experiment I seemed to decrease the probability that an egg was laid the following day. The absence of a long-term effect of experience from other parts of the study makes it seem unlikely that this was a learned response. Rather, it may have resulted from some form of oviposition fatigue as more moths laid eggs on the damaged rare patch than any other. In general however, I believe that the experimental design was inadequate to assess the importance of prior patch experience. The first patch composition experiment lacked the resolution to detect an effect of experience as the design was sensitive to the moths that did not respond. Further work on the question of prior patch experience would do better to focus on fewer patch types without the

complications of the cross-over design. It would seem that the disadvantages of this design far outweigh the promised improvement in the precision.

### **Larval experience**

According to Szentesi and Jermy (1990) there are two pre-conditions that have to be met to prove an effect of pre-imaginal experience on oviposition preference. The first is that there must be no possibility of emerging adults coming into contact with the larval food, either through direct contact or residues left on the cocoon – the so called ‘chemical legacy hypothesis’ (Corbet 1985). In the present study, removing pupae from the external cocoon, separating them from the remnants of the pre-pupal moult, and hatching them away from all hosts served to reduce this possibility. In addition, if there were a chemical legacy effect, there ought to have been a rearing plant effect on acceptance of intact cabbage because adult experience of cabbage was shown to increase its acceptability in Chapter 2 (Table 2.2) - no such effect was found (Table 3.7). The second condition is that there should have been no possibility of selection for genetically based preference in the insect lines used for testing. Test insects came from a single line that had been reared on rape for several generations and those that were conditioned on cabbage were only exposed to it for a single generation prior to testing. Therefore to explain the absence of any preference for damaged cabbage by cabbage reared insects in terms of a genetic difference between the two training groups, we would have to suppose that there was differential mortality of cabbage reared insects genetically pre-disposed toward preferring damaged cabbage. I find no reason to believe that this occurred.

A preference for damaged cabbage plants by rape reared insects came as a surprise in the absence of a similar effect in cabbage reared insects. The lack of an effect of rearing plant on the total number of eggs laid and the very small difference in pupal weights suggests that a difference in physiological condition cannot explain the observations. In addition, no effect of egg-load and pupal weight on discrimination between hosts was found for diamondback moth in Chapter 2. Therefore the most probable explanation is a systematic effect of larval experience, although it is not clear whether the observations resulted from a learned response or from changes in the chemosensory apparatus as have been observed in some larvae (Rogers & Simpson 1997; Bernays & Chapman 1998). Rogers and Simpson (1997) however, have suggested that chemosensory changes obtained during larval development are unlikely to be transferred to the adult stage in insects that undergo a pupal moult because of the associated remodelling of the cuticle.



It is not obvious from these data whether the apparent cross-induced effect is due to an increase in acceptability of damaged cabbage by rape reared insects, or a decrease in discrimination for damaged plants by cabbage reared insects. Jaenike (1983) has proposed that cross-induction occurs when experience on one host increases acceptance of another because of the chemical similarity between them. Even though the glucosinolate profiles of Brassicas differ between species (Lamb 1989), there is some evidence that these cues may be interpreted similarly. Apparently diamondback moths do not discriminate between individual glucosinolates with different side chains (Reed *et al.* 1989) and so it may be that cabbage and rape appear chemically similar to the moths. But it is still unclear how the absence of discrimination between glucosinolates transfers into discrimination between their hydrolysed products that are released following damage. However, chemical similarity between damaged states does not appear to explain why damaged cabbage plants were not preferred by cabbage reared moths. At the least we ought to expect that cabbage rearing should also trigger the response because it is the most similar stimulus to the test plants. Perhaps insects only develop an experience derived response toward intact plant cue profiles and that the preference by rape reared insects is because intact rape 'smells' like defended cabbage. This doesn't seem credible because the principle attractants in damaged *Brassica* plants are isothiocyanates which are almost absent from intact plants (Pivnick *et al.* 1994). Furthermore, if rape reared larvae learn, we might expect cabbage reared larvae to be capable of learning also and so prefer intact cabbage plants more than the rape reared moths did. No effect of rearing host on acceptance of intact cabbage was found (Table 3.7). Another possibility is that cross induction of preference for damaged plants did occur, but the stimulus was not intense enough in damaged cabbage to also induce this preference when reared on cabbage. Both associative learning and sensitisation are more likely when the conditioning stimulus is more intense (Abramson 1994). Further work would be required to elucidate this, perhaps using artificial diets laced with stimulatory compounds at different concentrations.

In the absence of cross-induction an alternative explanation for these data is that damaged cabbage are more attractive to diamondback moth provided the moth has not had previous experience of cabbage. If exposure to a cue found only in damaged cabbage induces some form of aversion or habituated response to damaged cabbage, then we might expect cabbage reared moths to show no preference for damaged cabbage. Many examples of larval habituation and aversion responses are known (for reviews see Szentesi & Jermy 1990; Bernays & Weiss 1996) but it is difficult to see how evolution could select for what would

effectively be inter-generation habituation. The nutritional benefits of diet diversification that can be accrued through larval habituation (Bernays & Weiss 1996) do not seem relevant to the choice of the initial larval host plant by the adult. Even habituated aversion to unpalatable foods does not seem to stack up when this process has seemingly occurred on a plant that is demonstrably suitable as larval food, as is the case with cabbage. It is possible that the change in preference that was detected is a hang-up from habituation as larvae, but Lee and Tseng (1993) found that larvae experience in diamondback moth enhanced larval preference for similar plants. Another possibility is that the damaged cues that are perceived by feeding larvae could be associated with the presence of other larvae. In this scenario the absence of preference for damaged plants could result from a conditioned aversion to cues that indicate the presence of other larvae. While numerous mechanisms exist by which adults can regulate the population density on single plants (Roitberg & Prokopy 1987; Dempster 1992; Blaakmeer *et al.* 1994; Schindek & Hilker 1996), I know of no examples in which adult behaviour shifts due to larval experience have been linked to particular host plants. A first step in testing this hypothesis would be to determine if larvae are deterred by the presence of other larvae. Overall, habituation would seem more likely if the reciprocal larval experience experiment were conducted, and insects tested on damaged and undamaged rape produced a reversal in the responses.

### Concluding remarks

In this chapter evidence has been presented that undamaged rare plants can escape herbivory by an adapted herbivore that is preferentially attracted to plants that have been previously damaged. It would seem that inducibility could lead to a lowering of the risk of herbivory for undamaged plants provided the undefended state is sufficiently rare. To take this work further, the next step experimentally would be to replicate this effect in a field situation to try and evaluate the fitness benefits to the plant population of this aspect of phenotypic plasticity.

From the insect's perspective, it is clear that preference for damaged *Brassica* could be a significant trade-off against the benefits of specialisation. It is generally assumed that specialised host location behaviour is a necessary adaptation to maximise the efficiency with which the most suitable hosts are located (Jaenike 1990). In this case however, larval concentration on a smaller fraction of the available plant resource appears to be a cost of the capability to discriminate between damaged and undamaged *Brassica*. In this regard it would be interesting to try and quantify the consequences of a failure to discriminate – would the

absence of discrimination result in an increasing likelihood of accepting a lower quality host? This is a difficult question to answer and may be spurious if the discrimination largely results from a stronger odour plume emanating from damaged plants, rather than a true preference. One approach however, may be to look at the response of sensory impaired moths to green leaf volatiles using the techniques of electro-physiology. Such techniques have been used to successfully identify cue-specific receptor sites on insect antennae (*e.g.* Hansson *et al.* 1999).

The phenotype discrimination picture is also complicated by the finding that the response to damaged plants is affected by larval experience. In this case, as cabbage reared moths preferred damaged cabbage to undamaged cabbage in the two-way choice test, but not in the larval experience no-choice tests, it seems that orientation to the stimuli is relatively unaffected by larval experience compared to post-alighting discrimination. Notably, Lee and Tseng (1993) and this study have both found no effect of larval rearing on preference for intact plants in diamondback moth. This clearly signals that damaged plants are a more sensitive test for larval induced preference and that the Hopkins host selection principle cannot be dismissed on the basis of tests with undamaged plants. Hopkins host selection principle raises interesting issues given the primacy of adult learning in oviposition acceptance. Oviposition acceptance based on larval experience involves a choice made on the basis of information that may already be out of date by the time it is used. Adult learning may provide a more immediate and accurate picture of the most appropriate oviposition acceptance decisions to be made. An important step for future work is to establish the degree to which adult experience modifies the behaviours derived from larval experience.

## CHAPTER 4

# THE EVOLUTIONARY STABILITY OF PLANT DEFENSIVE STRATEGIES AGAINST ADAPTED AND NON-ADAPTED HERBIVORES

### *INTRODUCTION*

Insect adaptation to plant resistance presents an evolutionary trade-off for plants. In the absence of resistance the plant is susceptible to all herbivores, however the expression of resistance can increase the cost of herbivory by adapted insects that use it as an attractant. In this regard it has been hypothesised that induced defence evolved as a means of reducing the cost of adapted herbivory, while still maintaining a resistance capability, by only deploying the defence when it is required (Karban & Baldwin 1997). The cost of adapted herbivory to a particular phenotype in this adapted herbivore escape hypothesis is dependent on the other phenotypes present within the population. A dependence of herbivore risk on the phenotype frequency relative to the alternative phenotypes was demonstrated experimentally in Chapters 2 and 3.

A number of studies have sought to model the evolutionary consequences of induced and constitutive defence strategies under various cost scenarios (Table 4.1). Cost is defined broadly in the sense of Karban and Baldwin (1997, p200) to include ecological (such as losses to adapted herbivores), as well as physiological costs of resistance. Two predictions are shared by most of the models. The first is that when the costs of being resistant are high or very low, constitutive non-resistant or resistant strategies respectively are favoured. This follows because for high costs, the marginal benefit of deploying any form of resistance is low. Alternatively, if resistance has very low costs, there is little fitness trade-off involved in its production. The second prediction is that variable environments seem to favour induced responses, provided the plant reliably adopts the state that maximises the plant's fitness under the particular environmental conditions that confront it. This latter condition has been termed predictability (Adler & Karban 1994) and in herbivore systems is usually modelled by varying the density of adapted to non-adapted insects.

**Table 4.1. Studies in which the evolution of induced resistance or tolerance has been modelled.**

Study	Resistance trade-offs	Frequency dependence of trade-off	Predictability and induction	Details and additional findings
Levins (1968)	Tolerance to different habitats	No	NA	Very general model on single and multiple phenotype strategies. Multiple phenotype strategies may be more fit if the upper right hand boundary of the "fitness set" (fitness set = set of possible fitness co-ordinates of single phenotypes in two or more environments) is concave.
Lloyd (1984)	Tolerance to different habitats has a structural cost	No	Induction favoured in coarse grained environments when the probability of accurately assessing the environment is greater than 0.5, and the structural costs of strategies are not too different.	ESS model in which plant structures are adapted to specific environments and carry a fitness trade-off in alternative environments.
Lively (1986)	Tolerance to harsh environments has a structural or material cost. Fitness cost when stress tolerant and non-tolerant morphs are in reciprocal habitats	No	Induction favoured when average probability of adopting the correct response is greater than 0.5	Constitutive strategies favoured when cost of tolerance are very high or very low
Clark and Harvell (1992)	Resistance to predation decreases growth and reproduction.	No	Non-adapted predator only	Optimal allocation model between growth, defence and reproduction. Conditional strategy favoured when predation is variable and at intermediate predator levels. Probability of attack has no effect on allocation strategy.
Till-Bottraud and Grouyon (1992)	Resistance to non-adapted herbivores decreases leaf growth rate	Semi-frequency dependent. Herbivore samples plants in patch until deterred by cyanogenic leaf. Rare acyanogenic plants able to invade optimal allocation to defence by cryptic association with cyanogenic morphs	Non-adapted herbivore only	Model of cyanogenesis in clover and trefoil. Cyanogenic morph favoured when high herbivore density

*Table 4.1 continued.*

Study	Resistance trade-offs	Frequency dependence of trade-off	Predictability and induction	Details and additional findings
Frank (1993)	Resistance to non-adapted herbivores decreases fecundity and increases death rate.	No	Non-adapted herbivore only	Optimises level of induced resistance in modified Lotka-Volterra system. High parasitic pressure and low costs of resistance favour induction. Sharp transition between uninduced and induced.
Adler and Karban (1994)	Resistance to non-adapted herbivores decreases the intrinsic growth rate of the plant.	No	Optimal induced defence favoured when herbivory highly predictable but variable. Constant herbivory favours constitutive strategy. Non predictable herbivory when there is a trade-off in insect specialisation favours "moving target model" - a random phase change model.	Level of defence is optimised. High costs to defence favour constitutive strategy. Variable herbivory at two density levels
Astrom and Lundberg (1994)	Resistance to herbivore decreases fecundity and survival	No	Unspecified	Level of defence is optimised. Induction favoured for high variation in herbivory.
Padilla and Adolph (1996)	Fitness cost when each of two morphs adapted to a particular habitat are in the reciprocal habitat. Physiological cost of adaptation in all habitats is not critical to model predictions.	No	Short time lags favour inducible strategy because better match with environment. High habitat transition probabilities decrease fitness of plastic phenotype.	Examines role of time-lags in induced response to randomly changing environments. Induction is bi-directional and responds without error to environment
Karban <i>et al.</i> (1997)	Cost of herbivory is negatively correlated with variability	Benefits to plant population at large are dependent on the states in which the other plants are. But benefit at the level of the individual is not dependent on the states adopted by the other members of the population.	Not applicable	Induction favoured if benefit to insect of plant resistance is a concave and negative function of chemical concentration. Model does not distinguish between variability due to induction or phenotypic variation between constitutive defended individuals.

Most existing models have assumed that the costs of deploying resistance are independent of the frequency of the resistance phenotypes. While this is a reasonable expectation for physiological costs to resistance, ecological costs such as herbivory may be strongly frequency-dependent because of the mechanics of herbivore host-choice behaviour (Papaj & Rausher 1983; Srinivasan et al. 1994; Smithson & Macnair 1997; Chapter 3 ). Frequency-dependent costs may have important evolutionary consequences for the strategies adopted by plants because the cost of adopting a particular strategy is dependent on the strategies adopted by the remainder of the population and may be different depending on the evolutionary success of a particular strategy. Till-Bottraud and Grouyon (1992) have shown that when a non-adapted herbivore feeds within a patch until a defended leaf is encountered, the optimal induced response is not an evolutionary stable strategy as rare undefended morphs can benefit from the protection of the patch and escape herbivory.

Herbivory by specialised insects is one particular form of ecological cost to defence. By definition, specialised insects are adapted to the resistance mechanisms of their hosts and will often use them as identifying cues (Ehrlich & Raven 1964; Nault & Steyer 1972; Renwick & Chew 1994). Hence there may be an ecological cost to defence manifest as increased predation by specialist herbivores. In terms of predictability, adapted insects make an environment less predictable for defended plants as adopting this state in an inappropriate response to the herbivore threat. If the herbivore threat comes from predominantly non-adapted herbivores then the threat is predictable. The ratio of adapted to non-adapted herbivores has been used as a measure of predictability in frequency-independent systems previously (Adler & Karban 1994). In the present study it is proposed to model a system containing non-adapted and adapted herbivores which make frequency-dependent host selection decisions. Thus we can determine whether frequency-dependent ecological costs

also favour induced resistance when costs are intermediate and highly predictable as found for frequency-independent systems.

### **TERMINOLOGY**

For simplicity, I will use the terms ‘defence’ and ‘resistance’ interchangeably to refer to a plant characteristic that decreases the fitness of non-adapted insects feeding on the plant, thereby increasing plant fitness. In contrast to the more restrictive use of the term ‘defence’ proposed by Karban and Myers (1989), I do not assume that being defended necessarily results in a net increase in plant fitness relative to being undefended. The ‘undefended’ phenotype is a plant defensive state such that all herbivores can feed on the plant without a fitness cost.

‘Constitutive’ refers to plants that have a single phenotypic state and will be used in conjunction with a descriptor referring to that state, as in ‘constitutive undefended’. ‘Inducible’ will refer to a plant that produces the defended phenotype when eaten by an insect. Sometimes a further descriptor will be used to refer to the phenotypic state of a group of inducible plants such as ‘inducible defended’. Three possible plant strategies will be examined in the context of this model: constitutive undefended, constitutive defended and inducible. For simplicity, I will assume that maternal strategies are inherited by the offspring.

I will be using the term ‘adapted’ to refer to an insect that is able to feed without a fitness cost on both ‘defended’ and ‘undefended’ host plants. The assumption that the defended phenotype is ineffectual against adapted insects also includes the absence of any deterrent value in the presence of the defence. The cues from defended plants are assumed to be at least as stimulatory to adapted insects as those from undefended plants. In this model ‘non-adapted’ will refer to an insect that feeds on undefended plants without a fitness cost, but



with a fitness cost should this plant become defended by induction. I have assumed that non-adapted insects are inhibited from laying any eggs on defended plants in keeping with the assumption that plants already in the defended state have perfect resistance to non-adapted insects. Preference of individual insects has been correlated with larval performance (Singer *et al.* 1988) and they will often refuse poor hosts if more suitable hosts are available (Wiklund 1981). 'Adapted' and 'non-adapted' are used in preference to 'specialist' and 'generalist' as these latter terms carry additional connotations regarding diet breadth.

### **OVERVIEW OF THE MODELS**

The model consists of a plant population whose range is stable, which is open to attack by a population of herbivores comprising an adapted (or partially adapted) herbivore species and a non-adapted herbivore species. A plant's phenotype may be either defended or undefended. These phenotypes may be obligate as in the constitutive defence strategies or conditional as in the inducible strategy. It is assumed that induced changes to the plant's phenotype do not affect the oviposition choices of females in the current time step and that between time steps, there is a fixed probability that an induced response will relax. As they die, plants are replaced via lottery competition in which the probability that a vacated site will be 'captured' by the offspring of a plant genotype is determined by its relative reproductive output of propagules which breed true with respect to the female defensive phenotype.

It is assumed that herbivores discriminate between host plants based on their defensive phenotypes and that acceptance decisions are phenotype frequency-dependent. Within host phenotypes, insects are dispersed randomly between individual plants. A mobile adult dispersal stage searches between plants and deposits immobile juveniles in a single clutch. A non-specified density-dependent process regulates herbivore density. Inter-specific interactions between the adapted and non-adapted insects are assumed to be negligible. A defended plant will reduce juvenile feeding and result in a decrease in adult fecundity in non-adapted and partially adapted insects.

### **THE MODELS**

A foldout reference table of the key symbols used in this chapter is given in Appendix C.

### A general plant population model

Let  $P_t$  be the proportion of cells occupied by plants adopting strategy  $A$  in a population also containing  $1 - P_t$  individuals who adopt strategy  $A'$ . I will assume that cells are left vacant when individuals of both strategies die according to a common death rate  $D$ . I will also assume that these vacant cells are captured by each strategy in the following time step in proportion to each strategy's abundance and  $k_t$ , the relative competitive advantage of  $A$  over  $A'$ . We can then describe  $P_{t+1}$ , according to a two-strategy lottery model:

$$P_{t+1} = P_t \left( 1 - D + \frac{k_t D}{k_t P_t + (1 - P_t)} \right) \quad (4.1)$$

(Pacala & Crawley 1992). When  $k_t = 1$  the model is neutrally stable. For  $k_t > 1$  the relative frequency of strategy  $A$  increases and when  $k_t < 1$  the relative frequency of strategy  $A$  decreases. In this model the relative success of each defensive strategy is determined by the cost of insect feeding on the reproductive output of each strategy. Thus we can define the competitive constant  $k_t$  as:

$$k_t = r_{A,t} / r_{A',t} \quad (4.2)$$

in which the  $r$ 's are the relative reproductive outputs of each strategy. In the absence of herbivory, I will assume that there is no trade-off in adopting a particular strategy so that  $r_{A,t} = r_{A',t} = 1$ . For constitutive strategists, the phenotype corresponding to the strategy is constant. This will not be the case for inducible strategists however, and so for convenience I will denote the general case for a plant's phenotype as  $x$ . I will also assume that the cost of herbivory, expressed in terms of the plant's relative reproductive output, is a negative

exponential function of herbivore load such as found for ragwort (Islam & Crawley 1983) and goldenrod (Root 1996). Such a cost function assumes weak compensation for damage at high herbivore densities. A physiological cost to resistance has not been assumed. A significant physiological cost of resistance to insects is not detectable in many plants (*e.g.* Simms & Rausher 1989, Karban 1993, Gianoli & Niemeyer 1997), and those that do have a cost may represent a minority of cases (Bergelson & Purrington 1996). I will also assume that the insect herbivores are sexually reproducing, lay a single clutch of eggs and have a sex ratio of 1:1. Hence the fecundity per female of genotype  $u$  is given by two times the average fecundity per insect (*i.e.*  $2F_u$ ). Assuming that herbivory from different herbivores has a multiplicative effect on plant fitness, we can write the expected relative reproductive output for a plant of phenotype  $x$ , with an average of  $m_{x,t}$  adapted, and  $g_{x,t}$  non-adapted insect herbivores per plant distributed independently of each other with probability  $\Pi_x(i)$  and  $\Pi_x(j)$  respectively, as:

$$r_{x,t} = \sum_{i=0}^{\infty} \sum_{j=0}^{\infty} \Pi_x(i) \Pi_x(j) e^{-2(F_m \theta_m i + F_g \theta_g j)} \quad (4.3)$$

where  $i$  and  $j$  are the number of adapted and non-adapted egg-laying female insects per plant respectively.  $F_m$  and  $F_g$  are the clutch size per insect for adapted and non-adapted insects and  $\theta_m$  and  $\theta_g$  give the respective scaling parameters for the costs to plants of herbivore feeding. According to equation (4.3), each plant of phenotype  $x$  has a probability equal to  $\Pi_x(i) \Pi_x(j)$  of receiving the eggs from  $i$  adapted and  $j$  non-adapted herbivores. Note that this formulation assumes spatial variation in the herbivore load between plants and variation between time steps for any given plant. Assuming that the probability generating functions are Poisson, equation (4.3) becomes:

$$r_{x,t} = e^{-(0.5(m_{x,t} + g_{x,t}))} \sum_{i=0}^{\infty} \frac{(0.5m_{x,t}e^{-2\theta_m F_m})^i}{i!} \sum_{j=0}^{\infty} \frac{(0.5g_{x,t}e^{-2\theta_g F_g})^j}{j!} \quad (4.3a)$$

Using the definition of  $e^x$ , equation (4.3a) can be written as:

$$r_{x,t} = \exp(-0.5(m_{x,t}(1 - e^{-2\theta_m F_m}) + g_{x,t}(1 - e^{-2\theta_g F_g}))) \quad (4.4)$$

The relative reproductive output for a particular strategy  $A$  is then given by the expected value of its constituent phenotypes. For a constitutive strategy expressing phenotype  $x$ ,  $r_{A,t} = r_{x,t}$ . For an inducible strategy containing phenotypes  $x$  and  $x'$ ,  $r_{A,t} = f_{x,t}r_{x,t} + f_{x',t}r_{x',t}$ , where  $f_{x,t}$  is the frequency of phenotype  $x$  at time  $t$ .

### Insect population dynamics

It will be assumed that competition among the relatively immobile larvae determines the number of individuals that survive to form the pool of mobile adult dispersers. This adult dispersal stage then determines the density distribution of larvae in the following generation. de Jong (1979) has described the population dynamics of a sexually reproducing insect population that lays a single clutch randomly between  $h$  cells as:

$$H_{t+1} = H_t F e^{-2cF} \exp\left(-0.5 \frac{H_t}{h} (1 - e^{-2cF})\right) \quad (4.5)$$

$F$  is the average fecundity per individual,  $c$  is a parameter controlling viability and  $H_t/h$  is the mean number of insect dispersers produced per plant. Equation (4.5) assumes that the mortality rate in any given cell is a negative exponential function of the density in that cell (i.e. mortality rate =  $e^{-2cFi}$  where  $i$  is the number of individuals in a particular cell). Note that the formulation of the equation does not explicitly suggest a spatial component to the distribution of the insects, although its derivation is based on individuals distributed between cells according to a Poisson process. As a consequence, the mean density is constant at equilibrium, but the insect density on any particular plant will vary between time steps. The

present model demands that insects reared at different mean densities on two plant phenotypes contribute to the common pool of dispersers. If we subscript for insect genotype  $u$  and plant phenotype  $x$ , and divide both sides of equation (4.5) by  $h_x$  plants, we obtain an expression for  $v_{x,t+1}$ , which is the expected contribution per cell of dispersers *reared* on plants of phenotype  $x$  to the global pool:

$$v_{x,t+1} = u_{x,t} F_u e^{-2c_u F_u} \exp(-0.5u_{x,t}(1 - e^{-2c_u F_u})) \quad (4.6)$$

$H/h$  has been replaced by  $u_{x,t}$  the expected number of insect dispersers *settling* in cells containing plants of phenotype  $x$  in the previous generation. The model is now no longer dependent on the number of cells and can be generalised to infinite cells as used in the plant model. For the Poisson distribution the mean is sufficient to define the probability distribution of insects dispersed among infinite cells.  $U_{t+1}$ , the global mean density of the insect dispersers across all plant phenotypes, is obtained by calculating a weighted sum of the mean dispersers per plant phenotype. Thus we have:

$$U_{t+1} = \sum f_{x,t} v_{x,t+1} \quad (4.7)$$

where  $f_{x,t}$ , as before, is the relative frequency of phenotype  $x$  at time  $t$ . The expected number of females that lay their egg clutch on plants of phenotype  $x$  is given by  $0.5u_{x,t}$  in equation (4.6). Given that the sex ratio is assumed to be 1:1, the expected number of egg-laying females will be given by half of the global mean density of all insects, multiplied by the probability that a plant of phenotype  $x$  is accepted for oviposition ( $E_{x,t+1}$ ), divided by the phenotype frequency. The half multipliers cancel out and  $u_{x,t+1}$ , may be written as:

$$u_{x,t+1} = \begin{cases} \frac{E_{x,t+1} U_{t+1}}{f_{x,t+1}} & \text{for } f_{x,t+1} > 0 \\ 0 & \text{for } f_{x,t+1} = 0 \end{cases} \quad (4.8)$$

### Host acceptance behaviour

The host acceptance model assumes that oviposition is triggered in part by the level of central nervous system excitation in response to specific experience on host plants. While it is based loosely on the Incremental Acceptance Model hypothesised in Chapter 2, it is intended to be a more general representation of frequency-dependent host acceptance involving learning. The level of excitation is modelled as a saturating function that is dependent on the rate at which insects encounter hosts, and the relative attractiveness of those hosts. Each host phenotype evokes an excitation response by the insect that is specific to that phenotype. It will be assumed that hosts are encountered at a constant rate, that there is no appreciable relaxation of the excitation state during the search period, and that experience on one phenotype does not affect the excitation state of another. I also assume that there is no effect of larval experience, and that the plant phenotypic states are constant during the host acceptance phase. Under these constraints we can define the rate of change of the excitation state  $Y_x$ , in response to encounters with hosts of phenotype  $x$  as:

$$\frac{dY_x}{dT} = s_x (y_{\max} - Y_x) \quad (4.9)$$

where  $s_x$  is the rate of host specific stimulation due to encounters with phenotype  $x$  and  $y_{\max}$  is the maximum excitation state. I use ' $T$ ' to denote a time scale occurring within a single generation as opposed to the between generation time scale that has been represented by ' $t$ ' in the remainder of the model. The rate of host specific stimulation is given by the product of the encounter rate and the stimulus intensity of the phenotype and may be written as:

$$s_x = \psi f_x I_x \quad (4.10)$$

where  $\psi$  is the search rate and  $f_x$ , as before, is the proportion of cells occupied by a host of phenotype  $x$ .  $I_x$  is the stimulus intensity of phenotype  $x$  and can take on a value between 0 and 1. Note that a proportional change in any of these constants yields the same effect as a proportional change in any other. It will be assumed that there is some excitation threshold value ( $y_{lay}$ ) required before a host phenotype can be accepted for oviposition. Response thresholds have been observed by Singer (1982, 1983) and they form the basis of the

hierarchy threshold model (Courtney *et al.* 1989; Courtney & Kibota 1990). The present model formulation however, differs from their interpretations in that the threshold is fixed and the excitation state changes. The time during the search period at which this threshold is reached is obtained by setting the initial value  $y_0 = 0$  and  $y_{max} = 1$ , substituting  $y_{lay}$  for  $Y_x$  and solving equation (4.9) for  $T$  to give:

$$T_x = -\ln(1 - y_{lay})/s_x \quad \forall s_x \neq 0^{\S} \quad (4.11)$$

$y_{lay}$  will be arbitrarily set at 0.5. After time  $T_x$  the insect is deemed to be 'responsive' to the phenotype and may lay eggs on it when encountered. The difference between the commencement of the responsive periods for two hosts gives the discrimination period during which only one host can be accepted.

Let the two host phenotypes within the present system be denoted by  $x$  and  $x'$ , to which the insect is responsive after  $T_x$  and  $T_{x'}$  respectively. The time at which both phenotypes are acceptable is given by:

$$T_{xx'} = \frac{T_x + T_{x'} + |T_x - T_{x'}|}{2} \quad (4.12)$$

We can calculate  $q_x$ , the proportion of the discrimination period that a host of phenotype  $x$  can be accepted as:

$$q_x = \begin{cases} 1 & \text{if } T_x < T_{xx'} \\ 0 & \text{else} \end{cases} \quad (4.13)$$

Let  $\beta$  be the probability that an insect does not accept a host during the discrimination period.  $\beta$  is equal to the probability that a fecund female will oviposit on a host plant that she has encountered, raised to the power of the total number of encounters with hosts during the discrimination period ( $n$ ). If the insects encounters hosts at a constant rate and accept hosts that they are responsive to with probability  $\Phi$ , then  $\beta$  may be written as:

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<sup>\S</sup> If  $s_x = 0$  then  $E_x = 0$ .

$$\beta = (1 - \Phi \sum q_x f_x)^n \quad (4.14)$$

where  $n$  is given by the discrimination period divided by the search rate or:

$$n = \frac{|T_x - T_{x'}|}{\psi} \quad (4.15)$$

The proportion of egg clutches laid on host phenotype  $x$  can be described as the proportion of clutches laid on the  $x$ 's during the discrimination period plus those laid post-discrimination following  $z$  encounters with potential host plants. Assuming that each insect lays a single clutch, and that  $f_x + f_{x'} = 1$ , this may be written as:

$$E_x = q_x(1 - \beta) + (\beta f_x \Phi + (\beta - \beta \Phi) f_x \Phi + \dots + \beta f_x \Phi (1 - \Phi)^{z-1}) \quad (4.16)$$

which can be rearranged to give:

$$E_x = q_x(1 - \beta) + \beta f_x \Phi \sum_1^z (1 - \Phi)^{z-1} \quad (4.17)$$

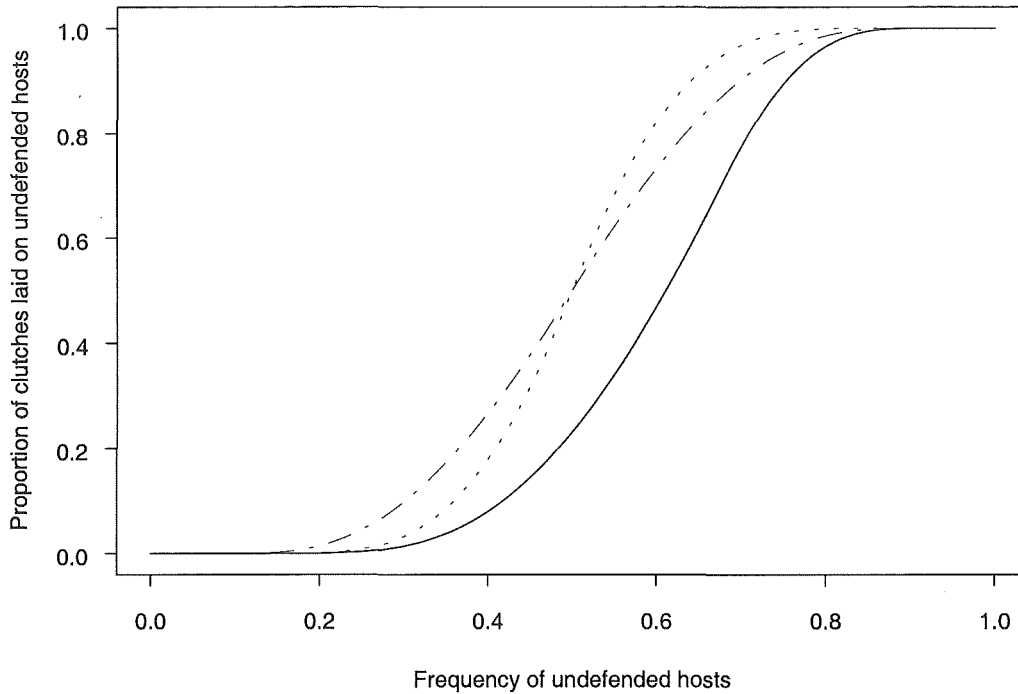
The summation is the sum of a geometric series with solution equal to  $1/\Phi$  for all  $\Phi < 1$ . In reality the total search period is finite, but as  $z$  gets large the proportion of insects who have not accepted a host becomes very small. Therefore we can obtain an approximate solution to this by allowing  $z \rightarrow \infty$ . Solving equation (4.17) and rearranging we obtain:

$$E_x = q_x - \beta(q_x - f_x) \quad (4.18)$$

Equation (4.18) is equivalent to:

$$E_x = \begin{cases} 1 - \beta(1 - f_x) & \text{when } x \text{ is preferred} \\ f_x & \text{when } x \text{ and } x' \text{ are equally preferred} \\ \beta f_x & \text{when } x' \text{ is preferred} \end{cases} \quad (4.19)$$





**Figure 4.1.** *Probability response curves for the host acceptance model. Alternate dash line:  $I_{\text{def}} = I_{\text{undef}} = 1$ ; dashed line:  $I_{\text{def}} = I_{\text{undef}} = 0.5$ ; solid line:  $I_{\text{def}} = 1$ ,  $I_{\text{undef}} = 0.5$ . Mutually high attractance levels and preferential attractance to defended hosts both decrease the sharpness of the transition in preference with increasing undefended host frequency. Other parameters:  $\Phi = 0.6$ ,  $\psi = 1$ .*

This model of host location produces a sigmoidal response function for the probability of accepting a host with increasing host frequency (Figure 4.1). The patch composition data in Chapter 3 also predict a non-linear relationship between relative phenotype frequency and the probability it is accepted for oviposition. Similarly, in field trials using marigold as a trap crop in tomato patches for *Helicoverpa armigera*, Srinivasan *et al.* (1994) found a non-linear relationship between the number of larvae on tomato and the relative frequency of tomato. Unfortunately their data do not have the resolution for high frequencies of marigold to determine whether this relationship is in fact sigmoidal. However, a sigmoidal acceptance function is a reasonable expectation if host plants are proportionally less likely to be accepted when rare than when common. A key feature of a sigmoidal response function is that if  $I_{\text{def}} \geq I_{\text{undef}}$ , then  $E_{\text{def}} \geq E_{\text{undef}}$  for all  $f_{\text{undef}} \leq 0.5$ . In other words, defended phenotypes when common, will always have a higher density of adapted insects than undefended phenotypes if they are more attractive. For extreme values of the general probability of

acceptance parameter  $\Phi$ , the sigmoidal response curve becomes linear. When  $\Phi \rightarrow 0$  then  $E_x \rightarrow f_x$ . For  $T_x < T_{x'}$ ,  $\Phi = 1$  gives  $E_x = 1$  and  $E_{x'} = 0$ .

This model assumes no minimum encounter rate for acceptance, and so there will always be non-adapted herbivores on undefended plants regardless of how rare the plants become.

### **GENERAL CONDITIONS FOR A STRATEGY TO BE EVOLUTIONARY STABLE.**

The conditions under which a strategy may be an evolutionary stable strategy (ESS) are set out in the following section. An ESS is a strategy such that when common it has greater fitness than rare alternative strategies. For simplicity, I will confine the analysis to situations in which the insect population dynamics are in a stable equilibrium giving invariable average costs to each plant phenotype. This analysis therefore excludes consideration of bet-hedging strategies that do better on average when the mean population density is more variable (Philippi & Seger 1989). Recall however, that the herbivore density at the individual level varies according to a Poisson process and therefore this assumption by no means implies a constant herbivore load per plant between time-intervals.

Consider a population consisting entirely of  $A$  strategists containing a single  $A'$  strategist. Let the fitness of a single  $A'$  strategist in a population of  $A$  strategists be given by  $W(A', A)$ , and the fitness of an  $A'$  strategist in a population consisting of  $\varepsilon A' + (1-\varepsilon)A$  strategists be defined as  $W(A'_\varepsilon, A_\varepsilon)$ . Then strategy  $A$  will be an ESS if for all  $A' \neq A$  either

$$W(A', A) < W(A, A) \quad (4.20a)$$

or

$$W(A', A) = W(A, A) \text{ and for small } \varepsilon, \quad W(A'_\varepsilon, A_\varepsilon) < W(A_\varepsilon, A_\varepsilon) \quad (4.20b)$$

(Maynard-Smith 1982). The plant lottery model assumes that the fitness of an individual is determined by its reproductive effort relative to all other members of the population. Therefore we can write  $W(A', A)$  as  $k_{x,b}$  the competition constant from the lottery model (equation 4.2), with  $W(A, A) = W(A', A') = 1$  by definition. For the most part I will focus on condition (4.20a), dealing with the special case where the fitness of both strategies are equal

only when considering invasion by constitutive defended strategists. This special case is considered in Appendix A.1.

### **EVOLUTIONARY STABILITY OF THE SET OF CONSTITUTIVE STRATEGIES**

Consider first a rare undefended mutant invading a population of defended strategists. Using the reproductive output equation (equation 4.4), the evolutionary stability condition (equation 4.20a) and assuming equilibrium insect population dynamics, we obtain a partial criterion for constitutive defended to be a pure ESS:

$$\begin{aligned} & \exp(-0.5(\hat{m}_{def} - \hat{m}_{undef})(1 - \exp(-2\theta_m F_m))) \\ & > \exp(-0.5\hat{g}_{def}(1 - \exp(-2\theta_g F_g))) \end{aligned} \quad (4.21)$$

$\hat{m}_{def}$  and  $\hat{m}_{undef}$  are the equilibrium densities of adapted herbivores on the defended and undefended phenotypes respectively, and  $\hat{g}_{undef}$  is the equilibrium density of non-adapted herbivores on the undefended phenotype. For a population of constitutive defended strategists, the density of non-adapted insects will be zero in the absence of immigration because we have assumed that the defence is perfect against these herbivores. In effect this is the same as considering a case in which herbivory is extremely unpredictable. Equation (4.21) can then be simplified to the requirement that  $\hat{m}_{def} < \hat{m}_{undef}$  for constitutive defended to be stable. For the adapted herbivores under consideration their response is limited such that undefended hosts are never more attractive than defended hosts and so there will always be a higher mean density of adapted insects on the common defended phenotype because the acceptance curve is a sigmoid function of host frequency. As such, the constitutive defended strategy is unstable. In this context the preferential attraction of adapted insects is an ecological cost of being defended when common. The inclusion of realistic immigration of non-adapted insects is unlikely to change this conclusion, as it would require very high

densities of non-adapted insects and/or a low plant population size to balance out the advantages of rare escape by undefended individuals. This is unlikely for several reasons. First, insects are much more likely to move out of patches containing poor quality hosts (Bach 1980; Karieva 1982). Second, if a population of non-adapted insects is maintained on reservoir hosts present within the same geographical location, under a frequency-dependent host acceptance model we would expect these alternative hosts to be accepted before the single undefended host. And finally, the Incremental Acceptance Model predicts that there is a minimum encounter frequency required before a host will be accepted. If this proves to be the case, then rare plants in large populations may not be encountered frequently enough to be accepted. The inclusion of a physiological fitness cost of resistance would also contribute to the instability of the defended strategy, because it would lower the fitness of defended plants.

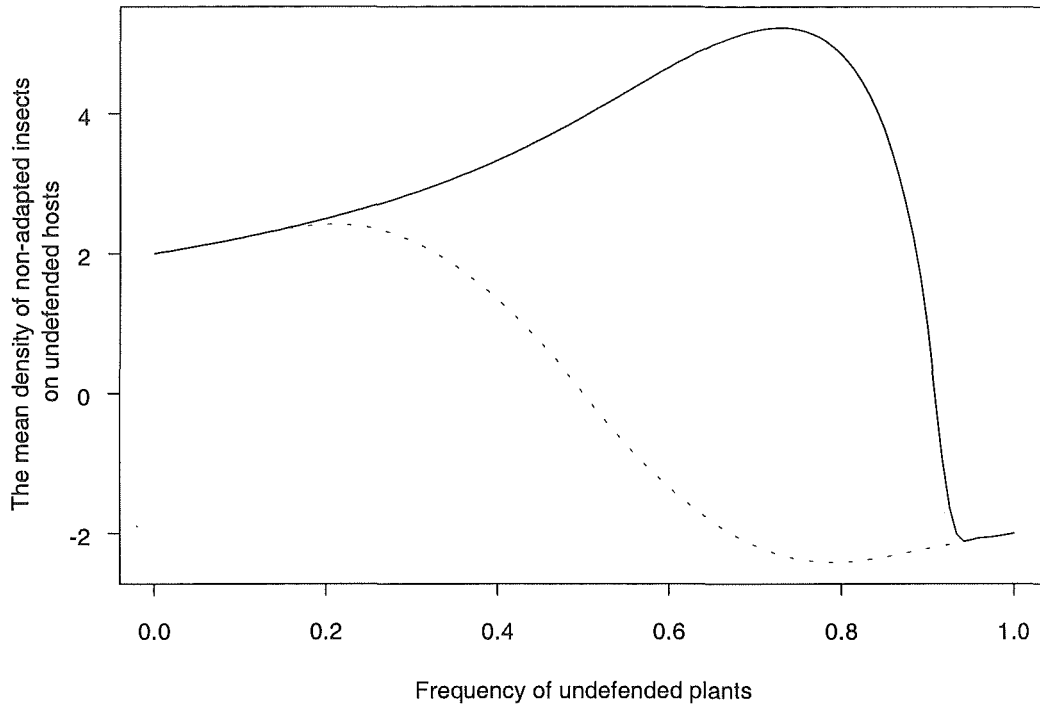
The conditions under which the constitutive undefended strategy can be a pure ESS are slightly more complex. In this case we again substitute the appropriate cost functions into equation (4.20a) and rearrange to give a similar equation to (4.21) but with the inequality reversed:

$$\begin{aligned} & \exp(-0.5((\hat{m}_{def} - \hat{m}_{undef})(1 - \exp(-2\theta_m F_m)))) \\ & < \exp(-0.5\hat{g}_{undef}(1 - \exp(-2\theta_g F_g))) \end{aligned} \quad (4.22)$$

The balance between non-adapted insects on undefended hosts and the difference between the adapted herbivore densities on defended and undefended plants determine stability. The undefended strategy becomes more likely to be stable as  $\hat{g}_{undef}$  becomes small. When  $\hat{g}_{undef}$  is minimised (*i.e.*  $\hat{g}_{undef} = 0$ ), the minimum requirement for the constitutive undefended strategy to be evolutionary stable is that  $\hat{m}_{def} > \hat{m}_{undef}$ . This latter condition becomes less

likely to hold the rarer the single defended mutant, because herbivore host choice is frequency-dependent. Thus the model predicts that if a population of constitutive undefended plants is sufficiently large, a single constitutively defended mutant can readily invade it. For small populations, stability is conditional on the distribution of adapted and non-adapted insects between host plants as determined by phenotype frequency (Figure 4.2). Note that if both host phenotypes are equally attractive to the adapted herbivore, then the constitutive undefended strategy is never an ESS because the rarer of the two hosts is always accepted less frequently than the common host. Maximal stability relative to non-adapted herbivore density occurs when  $\hat{m}_{def} - \hat{m}_{undef}$  is maximised. The frequency at which this occurs is determined by the proportion of eggs laid on each phenotype relative to their respective frequencies. Should both strategies be equally fit the evolutionary stability of the constitutive undefended strategy is stable provided the invader is rare (see Appendix A.1 for details).

In a two genotype system such as considered here for the constitutive strategies, a stable polymorphism comprising both strategies is always possible if neither strategy is a pure ESS (Maynard-Smith 1982). The frequencies of each strategy in the stable polymorphism are obtained by solving for  $r_{def} = r_{undef}$ . Of course the universal instability of constitutive defence in this case follows directly from the assumptions that the defence is completely effective against non-adapted herbivores and that adapted herbivores don't find undefended plants more attractive. Clearly the reverse result would be obtained if adapted herbivores found undefended plants more attractive, but this detail in itself is not important. What is significant, is that the ecological costs to each strategy change depending on their respective frequencies and that this generally promotes the coexistence of both constitutive strategies. Hence global densities of herbivores are less important than how they are distributed between the strategies.



**Figure 4.2.** *The maximum mean non-adapted herbivore density ( $\hat{g}_{\text{undef}}$ ) required for constitutive undefended hosts to be a pure ESS as a function of the frequency of undefended hosts. Negative values of  $\hat{g}_{\text{undef}}$  indicate where the strategy is always unstable. Solid line:  $I_{\text{def}} = 1$  and  $I_{\text{undef}} = 0.1$ ; small dash:  $I_{\text{def}} = I_{\text{undef}} = 0.5$ . All lines:  $\theta_g = \theta_m = 0.5$ ;  $F_g = F_m = 20$ ;  $M = 2$ .*

#### **INDUCED RESISTANCE AND CONSTITUTIVE DEFENSIVE STRATEGIES.**

We can add a third strategy to our existing strategy set. Consider the conditional strategy “inducible” in which host plants are induced to defend themselves when attacked by herbivores. When defended, inducible plants will appear to the ovipositing insects as having the same phenotype as the constitutive defended strategy. When undefended, inducible plants will appear as the constitutive undefended strategy with respect to oviposition decisions, although the two states are not identical due to the former strategy’s ability to mount a resistance response.

## Modifications to the model

We require several modifications to the model in order to accommodate the phenotype variability of the induced strategy.

### Frequency of undefended plants for an inducible population

In an inducible population, plants can adopt either the undefended, or the defended phenotype. Under the lottery model of plant dynamics, new individuals enter the population undefended with frequency driven by the death rate  $D$ . Plants become defended during the time step only if at least one adult female deposits her offspring onto them. It is assumed that feeding by adapted and non-adapted insects is concurrent and that the induced response reverts back to the undefended phenotype between time-steps with probability  $\lambda$ . The proportion of undefended plants in the next time-step is then given by the sum of the death rate, the proportion of surviving undefended plants that escaped herbivory and the proportion of surviving defended plants that spontaneously relaxed the response. We can thus write the proportion of host plants of the undefended phenotype as:

$$f_{undef,t+1} = D + (1 - D)(f_{undef,t} \Pi_{undef,t}(i = 0) \Pi_{undef,t}(j = 0) + \lambda(1 - f_{undef,t} \Pi_{undef,t}(i = 0) \Pi_{undef,t}(j = 0))) \quad (4.23)$$

As the insects are assumed to be distributed within plant phenotypes according to a Poisson process,  $\Pi_x(i = 0) = e^{-m_x}$  and  $\Pi_x(j = 0) = e^{-g_x}$ . Substituting for  $\Pi_x(0)$  into equation (4.23) and rearranging leads to:

$$f_{undef,t+1} = D + (1 - D)(f_{undef,t} \exp(-(m_{undef,t} + g_{undef,t}))(1 - \lambda) + \lambda) \quad (4.24)$$

It is easy to see that when  $m_{undef,t} + g_{undef,t}$  is large then the probability of escaping herbivory is very small and equation (4.24) can be approximated by the linear equation:

$$f_{undef,t+1} = D + (1 - D)\lambda \quad (4.25)$$

### Modifications to the insect population equations

It is assumed that constitutive defended hosts achieve perfect defence against non-adapted insects by preventing the laying of eggs and that inducible undefended plants are initially susceptible until induction occurs. It is also assumed that the induction of defence in undefended plants acts to reduce insect feeding, which in turn reduces non-adapted insect fecundity ( $F_g$ ). The fecundity of non-adapted insects in this case is dependent on how quickly a plant is induced. The induction delay is positively correlated with feeding damage. Let  $\tau$  be the induction efficiency parameter and assume that it is directly proportional to the induction time lag. Hence when  $\tau = 1$ , the defence response by a previously undefended plant is completely ineffective on the current generation of insects, and when  $\tau = 0$ , the response effectiveness is maximised to be equal to that of a constitutive defended strategist. The cost function for a non-adapted insect feeding on an inducible host shall be defined as

$$F_g = \frac{\tau \alpha F_{\max}}{\tau + \alpha - 1} \quad (4.26)$$

$\alpha$  is a parameter for the severity of the effect of defence deployment on the fecundity of non-adapted herbivores and must be greater than 1. The cost function is a saturating function of the induction efficiency parameter and has the property that when  $\tau = 0$ ,  $F_g = 0$ . Fecundity is maximised at  $F_{\max}$  when  $\tau = 1$ .



### The fitness of inducible strategists

The expected relative reproductive output of an inducible host when common is given by:

$$r_{inducible,t} = f_{undef,t} \exp(-0.5(m_{undef,t}(1 - e^{-2\theta_m F_m}) + g_{undef,t}(1 - e^{-2\theta_s F_s \tau}))) + (1 - f_{undef,t}) \exp(-0.5m_{def,t}(1 - e^{-2\theta_m F_m})) \quad (4.27)$$

which is simply the weighted sum of the expected reproductive output values for each of the two possible phenotypes. Note that the fitness term for the undefended phenotype also contains the defence efficiency parameter  $\tau$ . This parameter scales the potential non-adapted herbivore load to give the realised load according to how rapidly the induced defence is activated. When  $\tau$  is small the realised herbivore load is also small because rapid activation of the induced response reduces feeding by non-adapted herbivores. When invading a population of single phenotype strategists, equation (4.27) simplifies to:

$$r_{inducible,t} = \exp(-0.5(m_{undef,t}(1 - e^{-2\theta_m F_m}) + g_{undef,t}(1 - e^{-2\theta_s F_s \tau}))) \quad (4.28)$$

because new individuals enter the population undefended. As would be expected, single phenotype strategies invading a population of inducible strategists would experience the same expected density of herbivores as the corresponding inducible phenotype. The formula for the expected relative reproductive cost to constitutive strategists is therefore still given by equation (4.4).

Evolutionary stability of a two strategy systems containing induced resistance

### Constitutive strategies when common

When invading a population of defended strategists, the inducible strategy behaves much like a rare undefended strategy because it enters the population undefended, and is able to invade under the same conditions. Against a population of undefended strategists however, the inducible mutant initially appears to the insects as an undefended strategist. Consequently, the same mean density of insects that attack the undefended population will also feed on the induced mutant. Under these conditions, induction can invade a population of constitutive undefended plants because the fitness of the inducible invader is always more than that of the undefended strategy provided the value of the defensive efficiency parameter  $\tau$  is less than one and  $\hat{g}_{undef}$  is greater than zero. This follows because for  $\tau < 1$ , the induced defence decreases the non-adapted herbivore load in the current time step. In the absence of non-adapted herbivores, induction can still invade because if it induces during the current time step, the defended phenotype will escape herbivory in the following generation by being rare, provided the population is sufficiently large. And so the inducible strategy will be fitter than the constitutive undefended strategy. In summary, for non-zero adapted herbivore densities, neither constitutive strategy forms a pure ESS when the strategy set contains the inducible strategy.

### The inducible strategy when common

By substituting in the appropriate relative reproductive output values (equations 4.4 and 4.27) into equation (4.20a) and simplifying, the partial stability conditions for the inducible strategist against a rare constitutive undefended invader can be written as:

$$(1 - f_{undef,t}) \exp(-0.5(\hat{m}_{def} - \hat{m}_{undef})(1 - e^{-2\theta_m F_m})) < f_{undef,t} \exp(-0.5\hat{g}_{undef}(1 - e^{-2\theta_g F_g \tau})) - \exp(-0.5\hat{g}_{undef}(1 - e^{-2\theta_g F_g})) \quad (4.29)$$

Equation (4.29) states that the greater the potential benefit from being already induced (large  $\hat{g}_{undef}$ ,  $\theta_g$ ,  $F_g$ ,  $f_{undef,t}$  and  $\tau$ ) then the greater the relative fitness cost of attracting adapted insects to defended plants can be in order for induction to be an ESS. In other words, if the costs of defence are too high, plants with no ability to defend will be able to invade.

A similar equation can be obtained for a rare defended individual invading a population of inducible strategists and following simplification can be written as:

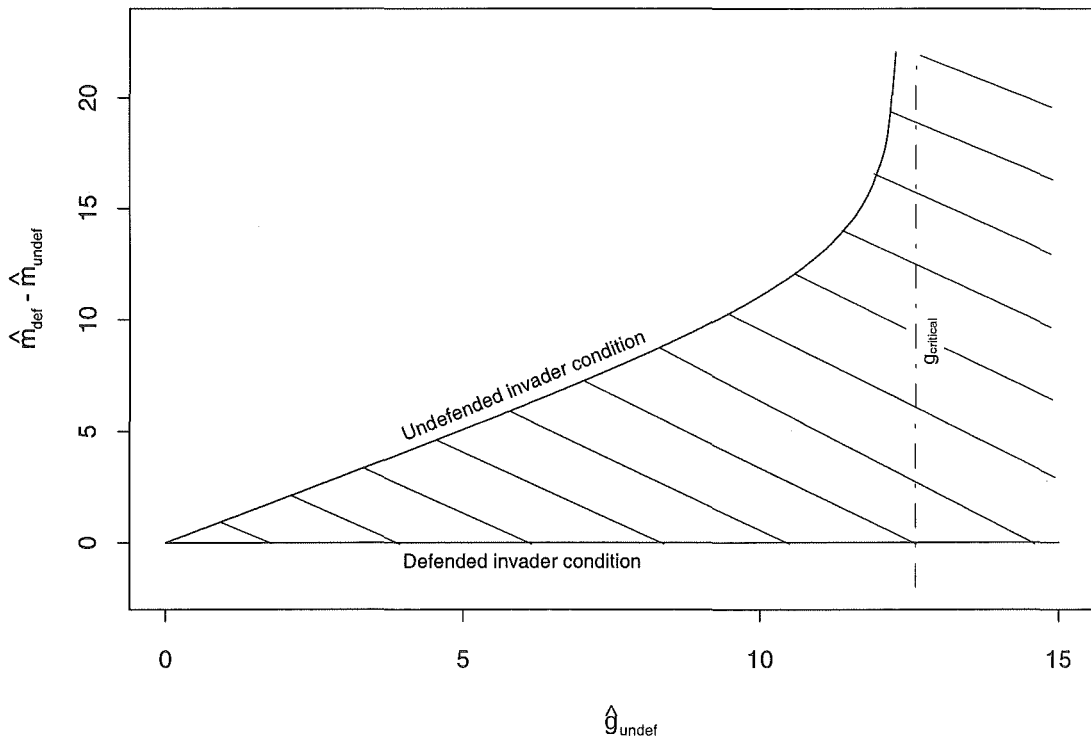
$$\frac{\hat{g}_{undef} (1 - e^{-2\theta_g F_g \tau})}{1 - e^{-2\theta_m F_m}} < \hat{m}_{def} - \hat{m}_{undef} \quad (4.30)$$

Equation (4.30) suggests that for inducible strategists to be stable against a constitutively defended strategist, then the benefits to defended hosts of escaping non-adapted herbivory must be less than the fitness cost of attracting adapted herbivores. If there is a high cost to the plant population of non-adapted insects (large  $\hat{g}_{undef}$ ,  $\theta_g$ ,  $F_g$  and  $\tau$ ) then  $\hat{m}_{def} - \hat{m}_{undef}$  must also be large for induction to be stable.

Equations (4.29) and (4.30) indicate that while too high a cost to defence makes induction invadable by undefended strategists, if defence carries insufficient ecological cost then selection favours constitutive defended strategists. Hence induction is favoured if the costs of being induced are intermediate. We can rearrange equations (4.29) and (4.30), to give the boundary conditions for induction to be an ESS in terms of the equilibrium densities of adapted relative to non-adapted insects:

$$\frac{\hat{g}_{undef} (1 - e^{-2\theta_s F_s \tau})}{(1 - e^{-2\theta_m F_m})} < \hat{m}_{def} - \hat{m}_{undef} < \frac{-2}{(1 - e^{-2\theta_m F_m})} \ln \left( \frac{\exp(-0.5 \hat{g}_{undef} (1 - e^{-2\theta_s F_s})) - f_{undef,t} \exp(-0.5 \hat{g}_{undef} (1 - e^{-2\theta_s F_s \tau}))}{1 - f_{undef,t}} \right) \quad (4.31)$$

These boundary conditions are shown graphically in Figure 4.3. Note that if the induced response is not defensive (*i.e.*  $\tau = 1$ ), the LHS of equation (4.31) equals the RHS and this condition no longer holds for any herbivore densities. The  $g_{critical}$  value in Figure 4.3 is the



**Figure 4.3.** A graphical representation of the ESS boundary conditions for induced resistance expressed as the difference between the adapted insect density on defended and undefended hosts ( $\hat{m}_{def} - \hat{m}_{undef}$ ) as a function of the density of non-adapted insects on undefended hosts ( $\hat{g}_{undef}$ ). The shaded region gives the necessary densities in order for induced defence to be an ESS. Below the upper boundary, induction is stable against an undefended invader. Above the lower boundary, induction is stable against the constitutive defended strategy.

asymptote for equation (4.29). For  $\hat{g}_{undef} \geq g_{critical}$  the induced strategy is always stable against undefended invaders because the contribution of inducible undefended plants to the fitness of the inducible population exceeds the fitness of the rare constitutive undefended mutant. This result arises because the frequency of induced plants sets a limit to the fitness costs to the inducible population of being defended. The equation for  $g_{critical}$  is obtained by allowing  $\hat{m}_{def} - \hat{m}_{undef} \rightarrow \infty$  in equation (4.29) and may be written as:

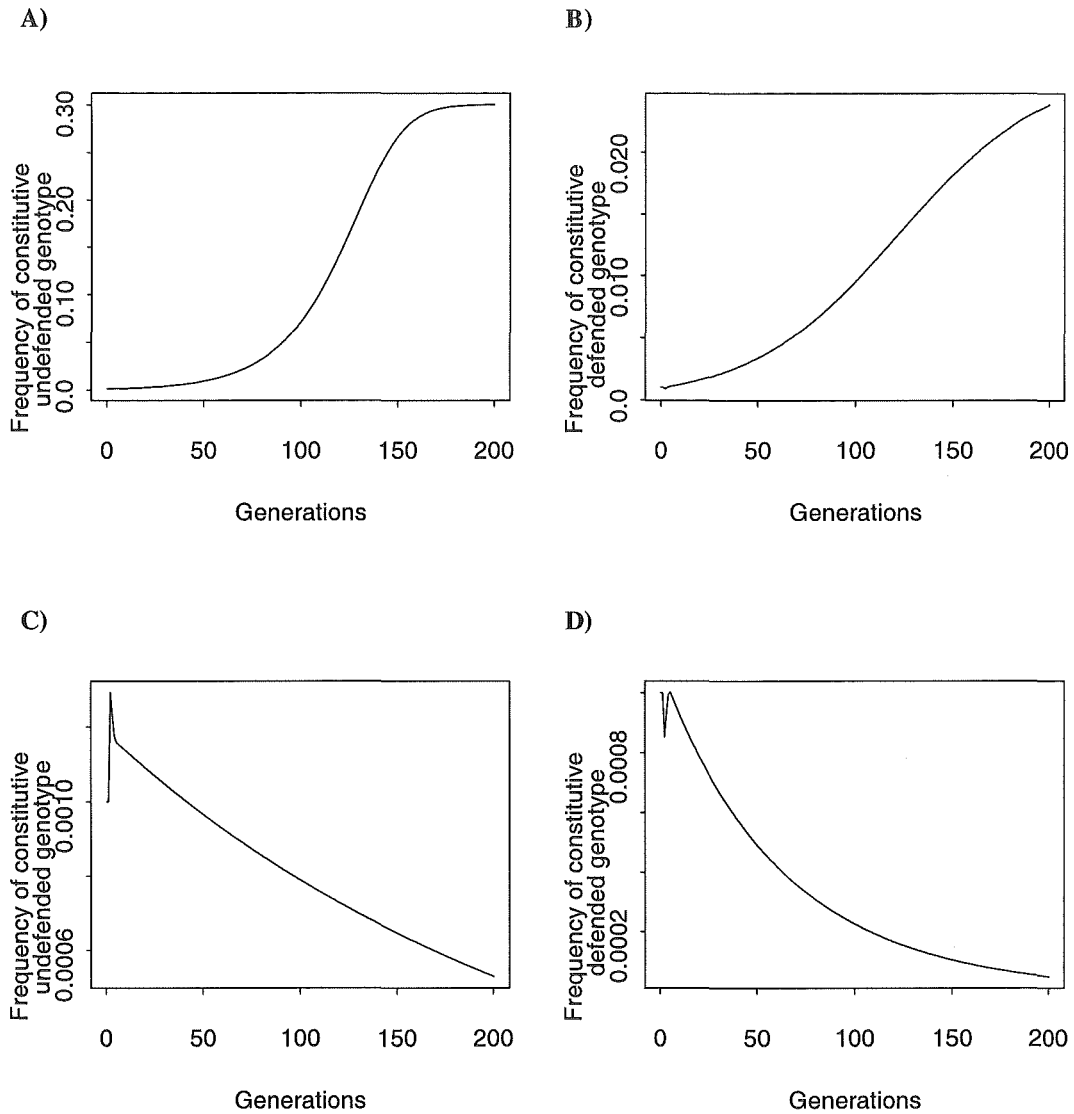
$$\exp(-0.5\hat{g}_{critical}(1 - e^{-2\theta_g F_g})) - f_{undef,i} \exp(-0.5\hat{g}_{critical}(1 - e^{-2\theta_g F_g \tau})) \leq 0 \quad (4.32)$$

If only to illustrate the mechanisms behind the evolutionary stability of induction, it is worth mentioning what the effect of the absence of either herbivore from the inducible system is on the stability of induction. From Figure 4.3, it is clear that if either herbivore species is absent, induction can only be an ESS under very restrictive conditions. If the non-adapted insect densities are zero, there is no benefit to being defended and as such induction can be stable only if  $\hat{m}_{def} = \hat{m}_{undef}$  and equation (4.20b) is satisfied. Alternatively when there is no ecological cost of being defended to balance out the defensive inefficiency of being inducible (*i.e.* adapted herbivores are absent) then induction is always invadable by the constitutive defended strategy provided the non-adapted herbivore density is not zero also. The value of the undefended invader condition, where defined, is always greater than that for the defended invader condition for all  $\tau < 1$  (see Appendix A.2 for proof). Therefore for every value of  $\hat{g}_{undef} \geq 0$ , there is an  $\hat{m}_{def} - \hat{m}_{undef}$  such that the induced defence is an ESS. When the induced response is no longer defensive ( $\tau = 1$ ), the upper and lower limiting lines are equal and no single strategy ESS is possible.

Under standard definitions of predictability, a high average global density of adapted herbivores relative to non-adapted herbivores gives an unpredictable environment for inducible plants if the response is ineffectual and carries a cost if deployed (Adler & Karban 1994). One of the requirements for evolutionary stability of induction is that the induced state cannot be fitter than uninduced inducible plants. This means that when the adapted herbivore density is greater than zero, there must always be more adapted herbivores on defended than undefended plants if induction is to be stable. In all conditions in which induction is an ESS, except when the fitness of the inducible phenotypes are equal, there is a net ecological cost of inducing due to these adapted insects. However Figure 4.3 shows that the evolutionary stability of induction is largely independent of the absolute global density of adapted herbivores that defines predictability. Instead, stability is a function of the relative distribution of adapted insects between phenotypes and this in turn is governed by the frequency-dependent host choice mechanism. This can be seen in Figure 4.4. In each simulation, the equilibrium global density of adapted herbivores is 2.6 to 2.8 insects per cell and that of non-adapted herbivores between 0.3 and 0.4 insects per cell. Clearly this is an unpredictable environment for inducible plants because there are approximately 6.4 – 8.3 times as many adapted herbivores as non-adapted herbivores. However, stability of the induced strategy is dependent on small changes in the distribution of adapted herbivores between the plant phenotypes due to their host selection behaviour.

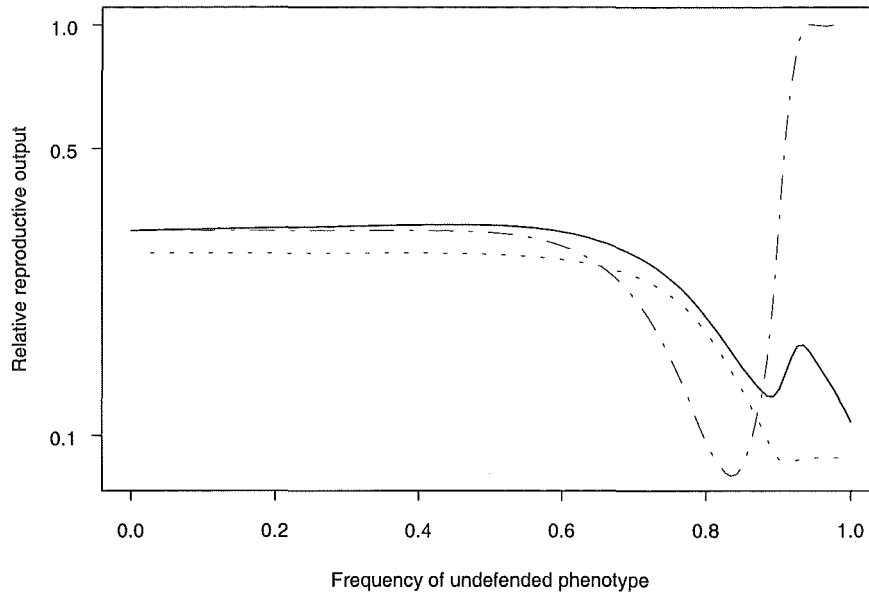
The importance of the interaction between phenotype frequency and the insect's acceptance behaviour to the evolutionary stability of the inducible strategy can be seen in Figure 4.5. At  $f_{undef} = 0$  the fitness of the common inducible and rare constitutive defended strategists are equal. As the undefended phenotype increases in frequency the constitutive defended strategist is able to invade (Figure 4.5a) because adapted herbivores feed more heavily on the

undefended phenotype. The undefended phenotype frequency at which this occurs is much

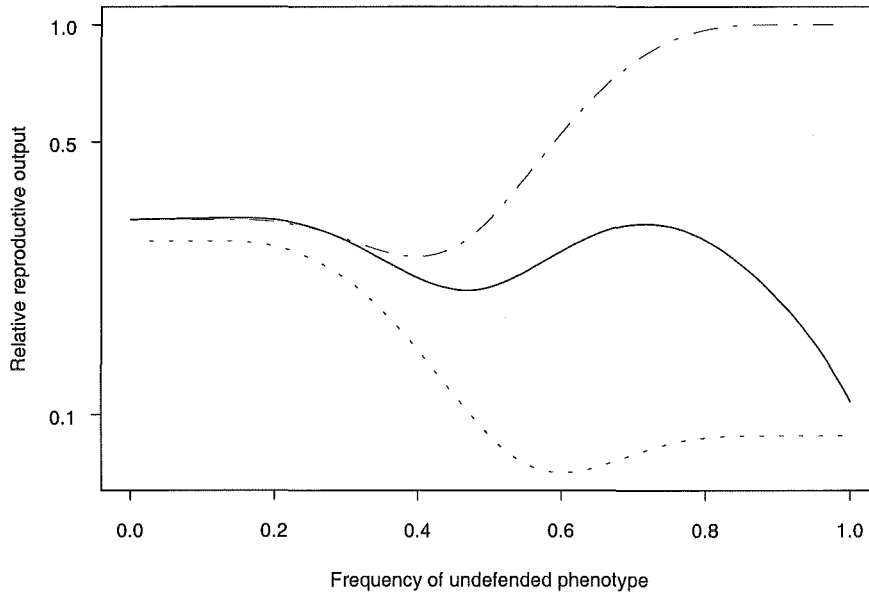


**Figure 4.4. Invasion trajectories of rare constitutive strategies within a population of inducible strategists.** For each simulation only the stimulus intensity parameters controlling host choice by specialist insects were varied. Each simulation began with equilibrium values for insect densities in a pure strategy inducible population. The initial frequency of the rare mutant was 0.001. A) Invasion by the constitutive undefended strategy.  $I_{def} = 0.8$ ,  $I_{undef} = 0.2$ . Initial values  $\hat{m}_{def} = 3.170$ ,  $\hat{m}_{undef} = 1.966$  and  $\hat{g}_{undef} = 1.064$ . B) Invasion by the constitutive defended strategy.  $I_{def} = 0.7$ ,  $I_{undef} = 0.3$ . Initial values  $\hat{m}_{def} = 2.962$ ,  $\hat{m}_{undef} = 1.840$  and  $\hat{g}_{undef} = 1.198$ . C) and D) Induction stable against constitutive undefended and defended strategists respectively.  $I_{def} = 0.6$ ,  $I_{undef} = 0.24$ . Initial values  $\hat{m}_{def} = 3.096$ ,  $\hat{m}_{undef} = 1.840$  and  $\hat{g}_{undef} = 1.198$ . All simulations  $D = 0.3$ ,  $\lambda = 0.5$ ,  $\tau = 0.1$ ,  $\theta_m = \theta_g = 0.5$ ,  $F_m = 20$ ,  $F_{max} = 30$ ,  $\alpha = 1.01$ ,  $c_m = c_g = 0.05$ ,  $\psi = 1$ ,  $\Phi = 0.6$ ,  $y_{lay} = 0.5$ .

a)



b)

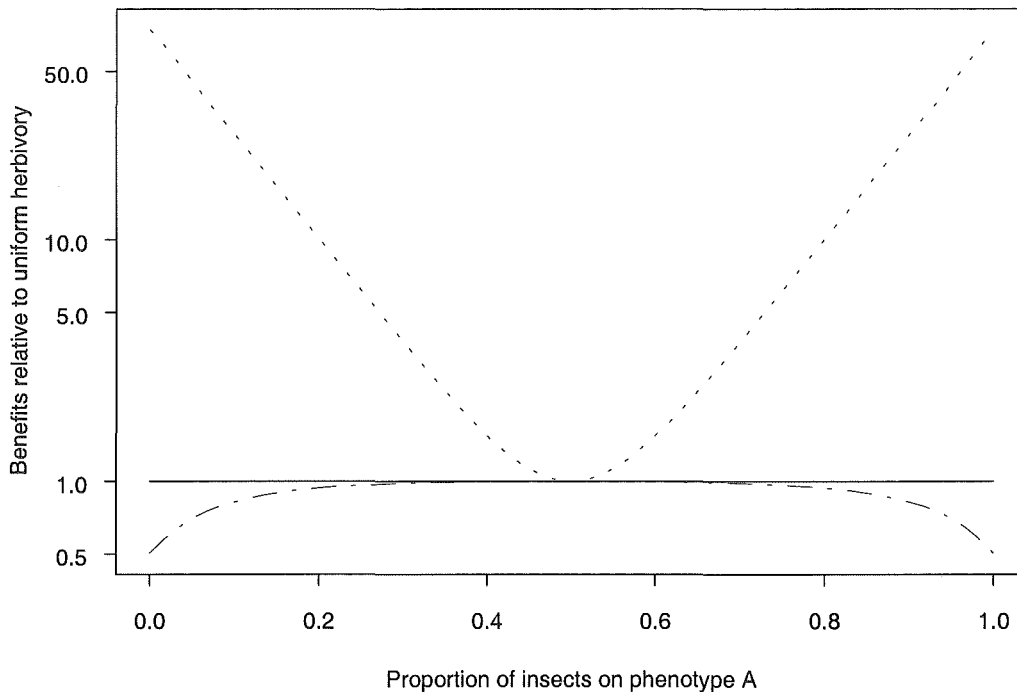


**Figure 4.5.** The dependence of the evolutionary stability of induction on the frequency of defended plants ( $f_{\text{undef}}$ ). Either constitutive strategy is able to invade when its relative reproductive output is greater than that of inducible strategists. For these simulations the rate of response decay is held constant at zero. Variation in  $f_{\text{undef}}$  is achieved by varying the death rate because the frequency of undefended plants is approximately linearly related to  $\lambda$  and  $D$  when  $\hat{m}_{\text{def}} + \hat{g}_{\text{undef}}$  is large (equation 4.28). The relative reproductive outputs are shown for induction when common (solid line), a constitutive defended invader (alternate dash) and a constitutive undefended invader (single dash). Maximum  $\hat{m}_{\text{undef}}$  is observed when the relative reproductive output of the constitutive undefended invader is minimised. Similarly, maximum  $\hat{m}_{\text{def}}$  is observed when the relative reproductive output of the constitutive defended invader is minimised. Parameters: a)  $I_{\text{def}} = 0.9$ ,  $I_{\text{undef}} = 0.1$ ; b)  $I_{\text{def}} = 0.5$ ,  $I_{\text{undef}} = 0.5$ ; All runs:  $\tau = 0.1$ ,  $\theta_m = \theta_g = 0.5$ ,  $F_m = F_{\text{max}} = 20$ ,  $\alpha = 1.01$ ,  $c_m = c_g = 0.05$ ,  $\psi = 1$ ,  $\Phi = 0.6$ ,  $y_{\text{lay}} = 0.5$ .



reduced when both phenotypes are equally attractive to the adapted herbivores (Figure 4.5b). The induced strategy is invadable by constitutive undefended strategies at intermediate values of  $f_{undef}$  and occurs when  $m_{def}$  is maximised relative to  $m_{undef}$  because the density of non-adapted herbivores is constant with  $f_{undef}$ .

The local minimum for the reproductive output for the common induced strategy at intermediate values of  $f_{undef}$  that was observed in Figure 4.5 represents the point at which the average cost of insects between phenotypes is uniform. This local minimum arises because of the direct benefits to the inducible strategy of unequal insect densities on each phenotype, resulting from the negative exponential cost function assumed in this model. This assumption means that the fitness loss to the inducible population of plants with a higher than average herbivore load, is less than the fitness gain from those plants with a lower herbivore load. Hence the fitness is higher than when the costs to each phenotype are equal. A linear cost function gives no benefit of distributing herbivores between host phenotypes and a convex function suggests a cost (Figure 4.6). Such observations are not new. Levins (1968) and Karban *et al.* (1997) have shown that provided the maximum level of constitutive defence is constrained, a concave cost function is sufficient to select for multiple phenotype defence strategies. In addition, Marquis (1992, 1996) has found that when the cost function is convex, those plants with herbivory concentrated on a branch have lower reproductive success than those with herbivory dispersed evenly throughout the plant. The choice of cost function does not however, alter the general conclusion that induction may be a pure ESS whereas constitutive strategies may not, because this result arises out of the relative distribution of insects between phenotypes. It will however, alter the relative differences in the fitness of the various defence strategies. Regardless of the cost function, there will also be a slight benefit overall of unequal insect distributions as unequal distributions decrease the total insect



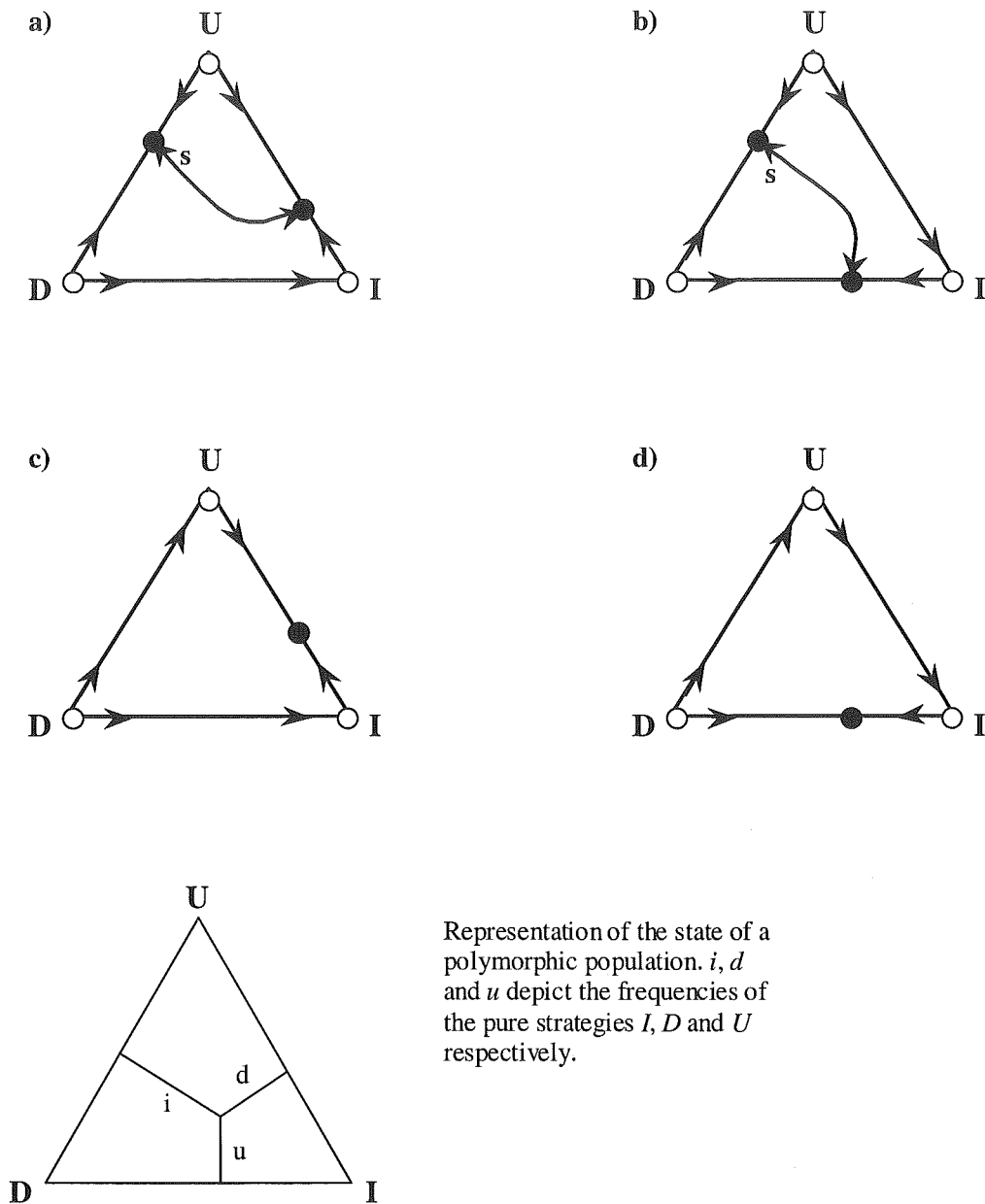
**Figure 4.6.** *The comparative reproductive output of a 2 phenotype strategy when herbivory is distributed unevenly between phenotypes, relative to uniformly distributed herbivory. It is assumed that the frequency of phenotype A is equal to that of A'. Various cost functions are given. A concave cost function assumes weak compensation for herbivore damage at high insect densities (dashed line,  $y = \exp(-x)$ ), a linear cost function assumes no compensation (solid line,  $y = \max - x/x_0$ ), and a convex cost function assumes strong compensation at low insect densities (alternate dashed line,  $y = \max - \exp(x - x_0)$ ). Parameters:  $\max = 1$ ,  $x_0 = 10$ ,  $x = 5$ .*

population density. This is due to the relatively more intense competition experienced by high densities of insects confined to a small part of the available plant resource relative to if they were distributed evenly throughout it.

Presently it is unclear whether the factors controlling phenotype frequency in inducible populations are under selection. If they were however, we might expect unconstrained selection on these factors to favour a phenotype frequency such that the fitness of both inducible phenotypes are equal. This situation corresponds to the defended invader boundary condition where the fitness of invader and common inducible strategy are equal. Under these

circumstances, induction is evolutionarily stable provided the undefended phenotype is rare (see Appendix A.1).

In the absence of a pure ESS, the model system will attain one of five possible stable states. The trivial case, when herbivory is completely absent, results in a neutrally stable polymorphism comprising all three strategies. The remaining possible states are limited, because only one of the two constitutive strategies is ever able to invade an inducible population for a given set of parameters. The invading strategy's identity depends on the parameter values, but the four possible stable states are identified in Table 4.2 and shown graphically in Figure 4.7. The first two scenarios in which the inducible strategy is invadable by either constitutive strategy each lead to one of two stable states, depending on the initial strategy frequencies. Both constitutive invaders may form a constitutive defended/undefended polymorphism or a polymorphism comprising itself and the inducible strategy (Figure 4.7a & b). For a very small population in which the constitutive undefended strategy is stable against a constitutive defended invader there is a single stable point corresponding to an inducible/constitutive invader polymorphism (Figure 4.7c & d). Clearly all of the stable states contain mixtures of the defended and undefended phenotype.



**Figure 4.7.** Schematic diagram of the flows for the games shown in Table 4.2. Solid black points indicate attractors and there is a saddle point at  $s$ . a) and b) indicate twin attractors - the eventual stable state will depend on the initial frequencies of the strategies. c) and d) indicate single stable states.

### **PARTIALLY ADAPTED HERBIVORES**

To date I have focused on adapted herbivores that have no fitness cost of feeding on the defended host and who find the defended phenotype at least as attractive as the undefended phenotype. We can extend the model by relaxing both of these assumptions in order to look

at the effects of partially adapted herbivores. As before, non-adapted herbivores are included in the model.

Consider a partially adapted herbivore whose maximum fecundity is reduced by a factor  $Q$  if reared entirely on defended hosts and whose fecundity is given by:

$$F_m = QF_{\max} + \frac{\tau\alpha F_{\max}(1-Q)}{\tau + \alpha - 1} \quad (4.33)$$

if reared on undefended inducible plants. Equation (4.33) is simply the minimum fecundity if induction is instantaneous plus the additional contribution to fecundity when there is a delay in induction, as determined by the induction efficiency parameter  $\tau$ . In keeping with the fitness cost to feeding on defended plants, I will also assume that this partially adapted herbivore finds undefended plants at least as attractive as defended plants (*i.e.*  $I_{\text{undef}} \geq I_{\text{def}}$ ). If insect fecundity is proportional to the feeding damage on a plant, then the cost of partially adapted herbivores on a plant of phenotype  $x$  may be written as:

$$r_x = \exp(-0.5\hat{m}_x(1 - e^{-2\Omega\theta_m F_m})) \quad (4.34)$$

For constitutive undefended hosts  $\Omega = 1$  (*i.e.* maximum cost to the plant). For constitutive defended and inducible defended hosts  $\Omega = Q$ . In the case of inducible undefended plants where the effectiveness of the defence is dependent on  $\tau$ ,  $\Omega = (Q + \tau(1 - Q))$ .

Using arguments similar to those used for the fully adapted insects, we can show that the stability of the constitutive strategies is reversed. A constitutive undefended population is always invadable by both constitutively defended and inducible phenotypes because of escape by the rare defended phenotype (following from the assumption that  $I_{\text{undef}} \geq I_{\text{def}}$ ) and the benefits to inducible undefended plants of deploying anti-herbivore resistance respectively. Recall that for constitutive defended populations we expect there to be no non-adapted herbivores. Hence, large populations of constitutive defended hosts may also be invaded by both alternative strategies due to escape from partially adapted herbivory by the rare invading strategy because both alternative strategies enter the population undefended. For smaller populations however, constitutive defended hosts can be stable because both alternative strategies will be sufficiently common as to be preferred by the partially adapted insects when  $I_{\text{def}} \ll I_{\text{undef}}$ . Note that a higher density of partially adapted insects laying on

undefended hosts is not required for the constitutive defended strategy to be stable. A slightly higher partially adapted herbivore load on defended hosts can in part be negated by the reduction in feeding efficiency brought about by the continuous deployment of the defence. If the density difference in favour of partially adapted herbivores on defended hosts is small enough, this reduction in feeding efficiency will elevate the fitness of the defended phenotype above that of the undefended phenotype and constitutive defence will be an ESS. High densities of non-adapted herbivores will also increase the probability that the constitutive defended strategy is stable. As might seem obvious, resistance that is effective against partially adapted herbivores tends to increase the stability of the defended phenotype and reduce that of undefended strategists. Note that a physiological cost to defence will have the opposite effect.

The stability conditions for the inducible strategy are predictably complex. The stability conditions for the constitutive undefended and defended invaders are given by:

$$\begin{aligned} & \exp(-0.5(\hat{m}_{undef}(1 - e^{-2\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_g F_g}))) < \\ & (1 - f_{undef}) \exp(-0.5\hat{m}_{def}(1 - e^{-2Q\theta_m F_m})) + \\ & f_{undef} \exp(-0.5(\hat{m}_{undef}(1 - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_g F_g \tau}))) \end{aligned} \quad (4.35)$$

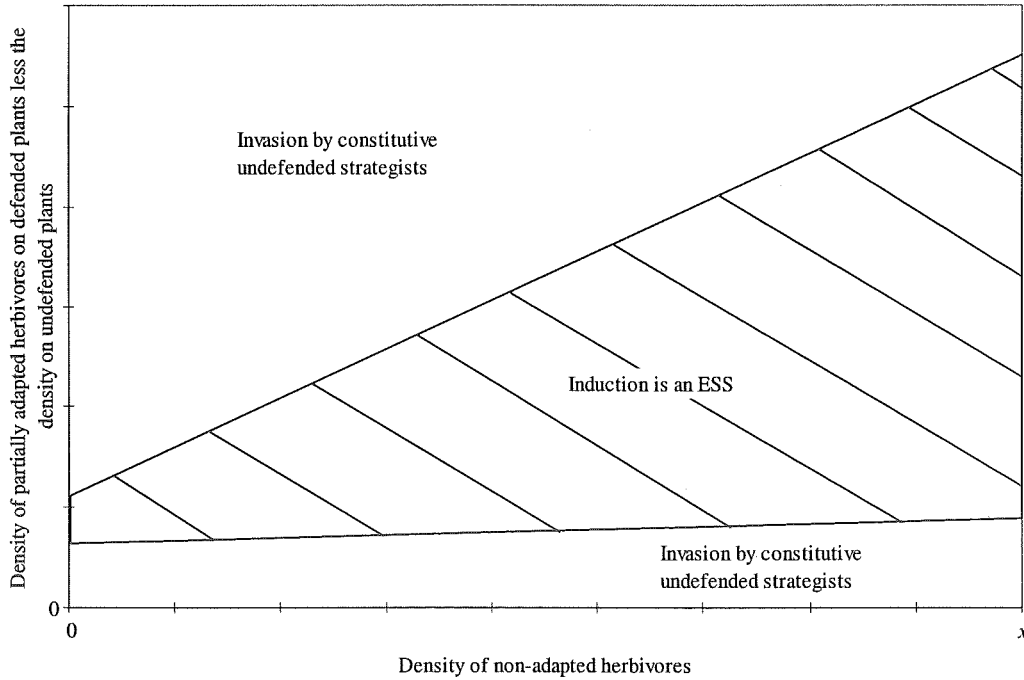
and

$$\begin{aligned} & \exp(-0.5\hat{m}_{def}(1 - e^{-2Q\theta_m F_m})) < \\ & \exp(-0.5(\hat{m}_{undef}(1 - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_g F_g \tau}))) \end{aligned} \quad (4.36)$$

respectively. When  $Q = 1$ , this model yields the same conditions as for the fully adapted herbivore model. Again by making  $\hat{m}_{def} - \hat{m}_{undef}$  the subject, the stability interval is written as:

$$\begin{aligned}
& \hat{g}_{undef} (1 - e^{-2\theta_s F_s \tau}) + \hat{m}_{def} e^{-2Q\theta_m F_m} - \hat{m}_{undef} e^{-2(Q+\tau(1-Q))\theta_m F_m} < \hat{m}_{def} - \hat{m}_{undef} < \\
& \hat{m}_{def} e^{-2Q\theta_m F_m} - \hat{m}_{undef} e^{-2\theta_m F_m} - \\
& 2 \ln \left( \frac{\exp(-0.5 \hat{g}_{undef} (1 - e^{-2\theta_s F_s \tau})) - f_{undef} \exp(-0.5(\hat{m}_{undef} (e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef} (1 - e^{-2\theta_s F_s \tau})))}{1 - f_{undef}} \right)
\end{aligned} \tag{4.37}$$

A significant difference from the previous model is that when  $\hat{g}_{undef} = 0$  the value of  $\hat{m}_{def} - \hat{m}_{undef}$  in the stability condition is greater than 0 for all  $Q \neq 1$  and  $\tau \neq 1$  (Figure 4.8). Furthermore the value of the undefended invader condition is always greater than the defended invader condition (Appendix A.2), and so in the absence of non-adapted herbivores induction can be an ESS for a range of  $\hat{m}_{def} - \hat{m}_{undef}$ . This results largely from the reduction in partially adapted insect feeding on defended plants. In the fully adapted insect model there was no reduction in feeding on defended plants. Therefore to satisfy both stability criteria, no



**Figure 4.8.** Schematic diagram showing ESS conditions for the inducible strategy for low densities (range 0 to  $x$ , where  $x$  is small) of non-adapted herbivores in the presence of partially adapted herbivores.

difference in adapted insect density on either host phenotype was required when  $\hat{g}_{undef} = 0$ . By relaxing this susceptibility condition, there must be more partially adapted herbivores on defended hosts relative to undefended hosts before the costs of herbivory are equal between the two phenotypes because of the more efficient reduction in the damage done by the herbivore on defended plants. Therefore the y-intercept for the constitutive defended invasion condition is greater than zero. And because the inducible undefended phenotype can reduce the damage done by the partially adapted herbivores, but the constitutive defended condition cannot, there can be an even greater density of partially adapted herbivores on defended plants before the fitness of both of these strategies are equal. Hence the y-intercept for the undefended invader condition is greater than that for the defended invader condition.

Polymorphic evolutionary stable states arise with the partially adapted herbivore in a similar manner to that for adapted insects. A key difference is that in Figure 4.7c & d,  $U$  and  $D$  are reversed when considering partially adapted herbivores. Note that in this case the population is small enough such that  $D$  is stable against  $U$  but unstable against  $I$ . If  $D$  is invadable by  $U$  then it is also invadable by  $I$ , but the converse is not always true. This follows because induction is an inherently better competitor than the constitutive undefended strategy when invading a constitutive defended population, since it can defend itself.

### **SUMMARY**

The evolutionary stability of constitutive and inducible defensive strategies has been examined within a context of non-adapted and adapted, or partially adapted, insect herbivores. The model assumes that herbivores choose between defended and undefended plant phenotypes according to the relative attractiveness and frequency of the phenotypes, and that they lay a single clutch of eggs. The distribution of herbivores between phenotypes determines the cost to a plant of adopting a particular phenotype. The insect's offspring are assumed to be relatively immobile and the larvae of non-adapted and partially adapted herbivores suffer a fitness cost when feeding on defended plants.

Under these assumptions a stable state always arises. Generally, all stable states contain both the defended, and the undefended phenotype. This result follows for the system containing non-adapted and adapted herbivores because the undefended phenotype escapes detection when rare, but constitutive undefended strategists are at a disadvantage relative to the inducible mutants when common, due to the latter's ability to defend itself against non-adapted herbivores. As a strategy containing both phenotypes, induction is the only pure ESS



obtained in large plant populations. If we admit the possibility of partially adapted herbivores, there is also an absence of single phenotype stable states in large populations. This result follows because while the undefended phenotype escapes herbivory when rare, the constitutive undefended strategy is at a disadvantage relative to the other two strategies when common because of their ability to defend against both non-adapted and partially adapted herbivores. If the population is small enough however, constitutive defended strategists can also be stable because the balance of partially adapted herbivores swings toward higher densities on the undefended phenotypes of the invaders. This, in combination with the more efficient defence against the partially adapted herbivores by the constitutive defended strategy, allows the strategy to be fitter.

Herbivore predictability is not a good indicator of the stability of the induced strategy as stability is determined by the distribution of adapted insects between phenotypes rather than their global density. Among the conditions for stability is the requirement that the undefended phenotype is at least as fit as the defended phenotype. Note that because both phenotypes can be equally fit and induction still remain stable, stability does not necessarily depend on group selection or additional constraints on the fitness of defended individuals. A lower fitness of the defended phenotype stabilises the inducible population against invasion by constitutive defended mutants. Provided the density of non-adapted herbivores is not too high, and the effectiveness of the induced response is sufficiently large, then the population will also resist invasion by constitutive defended mutants. There is a conflict in these two conditions in that, if the defence is too effective then the non-adapted herbivore density decreases such that the inducible strategy is once again invadable.

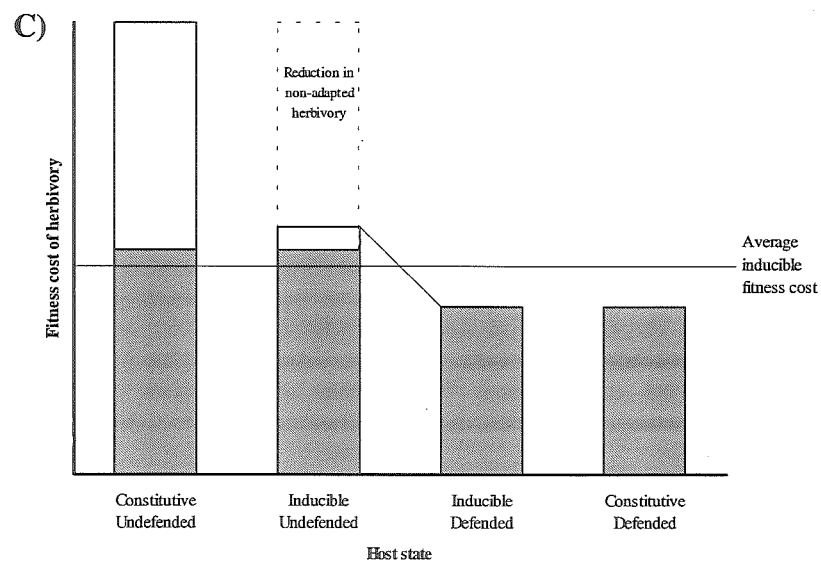
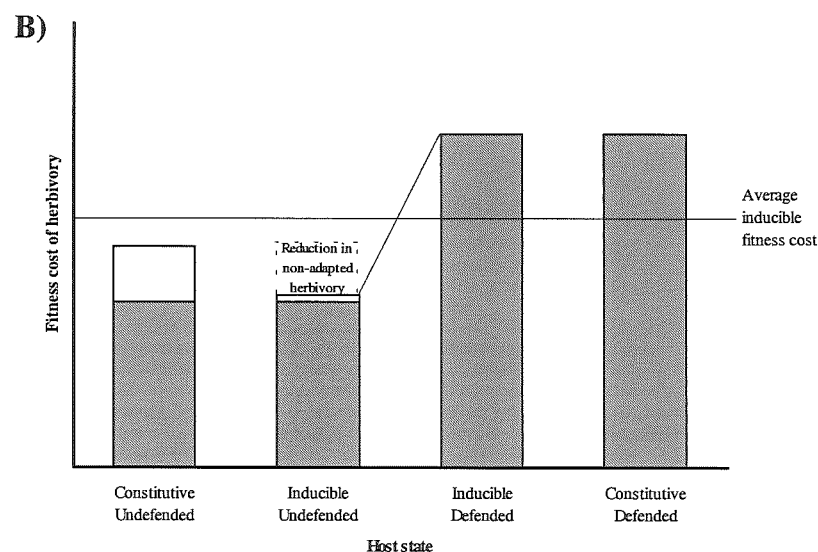
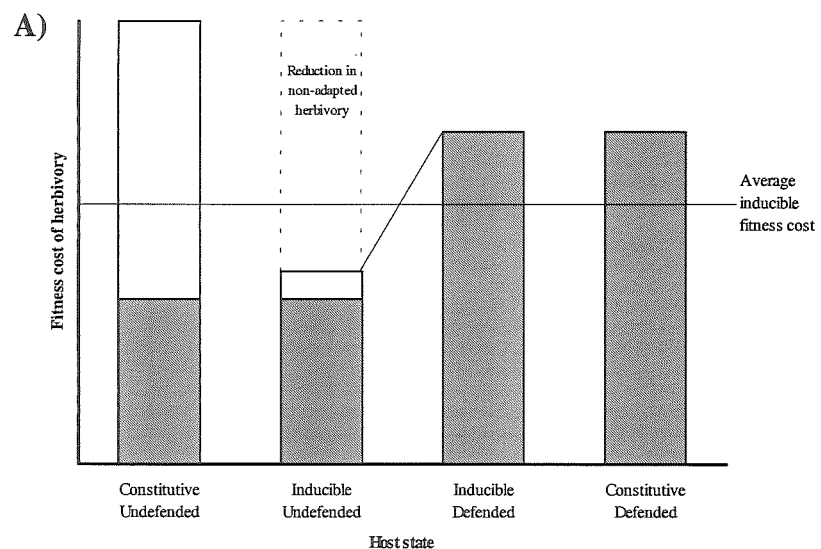
The phenotype frequency was shown to be critical to the stable state that is obtained because it determines the herbivore load on a particular strategy relative to the alternative strategies.

## CHAPTER 5

### FREQUENCY DEPENDENT HERBIVORY AND THE ADAPTED HERBIVORE ESCAPE HYPOTHESIS.

#### *PREDICTABILITY AND THE STABILITY OF THE INDUCED STRATEGY*

I demonstrated in Chapter 2 a dependence of oviposition choice on the encounter history of the insect with its host plants. The more experience that an insect had with a host plant, then the more likely it was that the plant would be accepted on subsequent encounters. The rate at which experience is acquired is affected by the probability of encounter. Host frequency is correlated with encounter probability (Mackay 1985; Smithson & Macnair 1997) and a dependence of acceptance probability on host frequency was illustrated in Chapter 3. A plant phenotype was much more likely to be accepted when common than when rare, but only undamaged plants when rare were less likely to be laid on than if eggs had been laid randomly with respect to plant phenotype. In this case however, it seemed that pre-landing orientation to host-plant odours was primarily responsible for mediating the observed preference because insects accepted both host phenotypes with equal probability when they were presented in a no-choice situation (Table 3.7, see discussion Chapter 3). Having developed this theme of frequency-dependent oviposition acceptance, the consequences of such behaviour have been applied more formally to the evolution of plant defensive strategies in the absence of a physiological cost to defence. The model in Chapter 4 considered induced resistance, constitutive resistance and constitutively undefended as the three possible plant defence strategies. Under selection by non-adapted and adapted, or partially adapted, herbivores, a stable state was always obtained. All stable states under an adapted/non-adapted herbivore regime contained a mixture of defended and undefended plants - there were no constitutive pure evolutionary stable strategies because induction was able to invade both constitutive strategies under the conditions assumed in the model. As the only strategy capable of expressing both the defended and undefended phenotype, induction was the only pure ESS. It was found that the evolutionary stability of induced defence depended on the distribution of herbivores between the two phenotypes (Figure 5.1). Similar results were obtained for large plant populations under selection by a non-adapted/partially-adapted herbivore population. In small populations however, constitutive defended strategies may also be stable. This qualitative difference in the stable states obtainable under



**Figure 5.1.** A schematic diagram showing the evolutionary stability of a common induced strategy under several herbivore regimes. Induction is an ESS if its total fitness cost of herbivory is lower than that of the two alternative strategies. Shaded bars indicate the fitness cost to each strategy of herbivores adapted to the induced state. Clear bars with solid border indicate the fitness cost of non-adapted herbivory. The clear bars with dashed border show the reduction in non-adapted herbivory from that expected on an undefended plant due to the induction of resistance during feeding. A/ Induction is an ESS. B/ Induction can be invaded by a constitutive undefended individual due to low non-adapted insect densities. C/ Induction can be invaded by a constitutive defended individual due to a low cost of adapted herbivory on defended plants.

adapted versus partially adapted herbivores is largely due to the susceptibility of the partially-adapted herbivore to defended plants. Recall that non-adapted herbivores were assumed to be completely susceptible and so were not represented in a habitat comprising only constitutively defended plants. Partially-adapted herbivores were assumed to be partially susceptible and adapted herbivores non-susceptible. Constitutive resistance is widely recognised as being more effective at reducing herbivore damage than inducible resistance because for induction there is a delay between receiving the cue to induce and mounting the induced response (Karban & Baldwin 1997; Baldwin 1998). First let's consider a situation in which a constitutive undefended individual invades a constitutive defended population that is under selection by a partially adapted herbivore. The defended strategy will be unstable provided the realised herbivore load on defended plants (obtained from the actual herbivore load less the reduction in damage through being defended) is greater than the herbivore load on undefended plants. In small populations, a single undefended mutant would be sufficiently common such that the herbivore load on the undefended plants would be greater than the realised herbivore load on the defended plants because of frequency-dependent host choice by the herbivores. Hence in these small populations the constitutive defended strategy would be stable. A similar argument may be offered for a rare inducible mutant, except that because it too can defend itself, the population size at which its realised herbivore load exceeds that of the constitutive strategy is much smaller. The constitutive defended phenotype is more likely to be stable if the undefended phenotype is much more attractive to the insect relative to the defended phenotype. This situation did not arise in the system under selection by adapted herbivores because the adapted herbivores were constrained such that they never preferred the undefended phenotype when rare. There was also no direct benefit to a plant deploying the defence against the adapted herbivores.

It is generally predicted that very high or very low costs of producing the defended phenotype tend to favour constitutive undefended and defended strategies respectively (Lloyd 1984; Lively 1986; Clark & Harvell 1992; Frank 1993; Adler & Karban 1994; Åström & Lundberg 1994; Padilla & Adolph 1996). The current study found agreement with these predictions in as much as the ability of constitutive strategies to invade an inducible population appeared to follow this pattern, however because of the frequency-dependence of the cost this did not generally result in the evolutionary stability of the constitutive strategy. The adapted herbivore escape hypothesis proposes that attraction of adapted herbivores is the cost of producing resistance. Unlike a physiological cost however, the cost is experienced only as a result of continuing to defend rather than the act of producing the defence itself because only

the former carries the added risk of preferentially attracting adapted herbivores. Hence induction, followed by rapid relaxation once the non-adapted herbivores are deterred, will carry minimal cost. A high cost to producing an induced response in this context would mean a high density of adapted herbivores on the defended phenotype relative to the undefended phenotype. Under these circumstances we might expect a rare constitutive undefended strategy to increase and a constitutive defended mutant to go extinct. But the cost of adopting a particular phenotype is frequency-dependent, and so a constitutive undefended phenotype when common would experience a high herbivore load relative to a defended invader under selection by the same herbivore population. In this case the stable state is either a constitutive defended/undefended, or constitutive undefended/inducible polymorphism (see Fig 4.8a). By a similar argument it is possible to show that when the cost of adapted herbivory is low on the defended phenotype relative to the undefended phenotype, then the inducible population is invadable by the constitutive defended strategy. But when common, the constitutive defend strategy is invadable by both alternative strategies leading to a constitutive defended/undefended, or constitutive defended/inducible polymorphism (see Fig 4.8b). Therefore in the current model the cost of resistance is replaced by a cost of adopting a particular phenotype relative to the alternative. Because of this frequency dependence, the expectation that an optimised constitutive strategy can be stable with constant herbivory across phenotypes (Clark & Harvell 1992; Adler & Karban 1994) did not arise in this model because of the potential for escape by adopting a different phenotype. Even when the rules for the distribution of herbivore costs were changed to allow partially adapted herbivores, there was little qualitative change to this prediction. The exception was in small plant populations where constitutive resistance can also be an ESS. Frequency dependence is a more realistic representation of the risks of herbivory and is a significant omission from previous models.

The expectation that induction is only favoured when predictability is high (Lloyd 1984; Lively 1986; Adler & Karban 1994; Padilla & Adolph 1996) was also not supported in the present study. We can define predictability as the probability that the defensive response, or state, correctly matches the identity of the stress - in the case of the current model it is the herbivore attacker. Therefore high densities of adapted herbivores give low predictability for inducible strategists because either inducing if undefended, or remaining induced, is an inappropriate response. Lloyd (1984), Lively (1986) and Padilla and Adolph (1996) all use a similar argument to predict that induction is favoured only when predictability is high. Essentially their systems contain two host states that are each adapted to their respective

favoured environments, but have a lower fitness when growing in the preferred environment of the alternate state. In a system containing two environments, the long-term average frequency of at least one of the environments will be  $\geq 0.5$ . Assuming that the cost of occupying the wrong environment is similar for both plant states, induction will be the fittest strategy if it adopts the phenotype that maximises fitness when encountering either environment with probability greater than the average frequency of the most common environment. If induction does not, the constitutive strategy that best matches this common environment will be the most fit. The argument of Adler and Karban (1994) is similar except that in addition to constitutive strategies, they model both unidirectional (always switch to the defended state when eaten) and random bi-directional (switch randomly to any state) inducible strategies, all under selection by herbivores. In the present study, no generalisations about the effect of the global density of adapted herbivores (and consequently predictability) on the stability of induced resistance were possible. The stability of the inducible strategy depended on the difference in the adapted herbivore density between the two plant phenotypes rather than the global density. This is not the same however, as saying that the stability of the inducible strategy is independent of global density. If there is a fixed probability that the adapted herbivores will establish on each phenotype, the absolute difference in density between the phenotypes will increase with increasing global density. But this effect of increasing global density can be stabilising, or destabilising, depending on the initial difference between phenotypes. In part, this result arises because of the absence of a cost to an individual in the current generation of switching to a defended state in the face of low predictability. As such, switching to the defended state in the current time step is always a good option regardless of the density of the adapted herbivores, because the herbivore load is already determined. Of course, remaining defended carries a cost to the individual, but stability of the inducible strategy requires some induced individuals to be present otherwise the strategy is invadable by an constitutive defended strategist. All of the previous models assume a fitness cost of switching to the defended state in an unpredictable environment, either in terms of a simple growth penalty due to physiological costs, or by making the plant more susceptible to feeding by a defence adapted herbivore. In the current model, a physiological cost to inducing has been deliberately omitted, and switching to the defended state does not increase the damage done by an insect already present on the plant. By assuming a trade-off in insect fitness on undefended plants of specialisation on defended plants, such as adopted by Adler and Karban (1994), the cost of switching to the defended state is inflated because the undefended state is also effectively resistant. I don't believe that such a trade-off is justified for the situation under consideration. In the first instance, the

model simulates rapid induced resistance in which induction affects the herbivores that were responsible for inducing the plant. Therefore adapted insects will experience an induced environment for much of their lives anyway if the time delay is short, and so any trade-off is minimal. Secondly, adaptation to an induced response need not carry a fitness trade-off on undefended plants (Scriber 1978; Anderson & Alborn 1999). In fact many of the cases in which increased insect fitness has been found following induced changes to plants, cannot arguably be considered resistance responses anyway. In most of these cases, there is either an increase in the nutritional value of the plant in the absence of a deterrent effect on non-adapted insects (English-Loeb & Karban 1991), or insect feeding promotes regrowth of younger, more palatable tissue (Webb & Moran 1978; Pullin 1987; Potter & Redmond 1989; Messina *et al.* 1993). For example, the specialist herbivore *Aglas urticae* grew faster and larger, and ate less food on re-growth leaves following artificial defoliation of *Urtica dioica* (Pullin 1987). Re-growth leaves were associated with increased total organic nitrogen, increased soluble protein, and increased insoluble nitrogen, but lower water content. There may be no benefit for plant fitness of a trade-off in insect fitness when on uninduced plants where this is due to increased nutritional quality on defended plants. As has been pointed out by Leather and Walsh (1993), often insects feeding on nutritionally poor tissue end up consuming more of the plant in order to fulfil their developmental requirements.

### **PHENOTYPE FREQUENCY AND HERBIVORY RISK**

The relative frequency of undefended inducible hosts as a determinant of herbivory risk has been shown in this thesis to be a key factor in the evolutionary stability of induced resistance. If undefended hosts are too common, constitutive defended hosts are able to invade. If too rare, then constitutive undefended hosts can increase. So what determines the phenotype frequency? Where seasonal variation can be identified, the change in the frequency of undefended hosts from the end of one season to the beginning of another is largely determined by the relaxation rate of the response and new individuals entering the population. Even at fairly modest insect densities, the probability that a plant escapes damage is low and so is probably not important unless the herbivore risk is also low. Of these generating mechanisms, relaxation is the only one that can be expected to be under some selection for its role in defence. Currently we do not know whether there is a heritable genetic basis to phenotype frequency. The degree of inducibility appears to be genetically determined (Maddox & Root 1987; Zangerl & Berenbaum 1990; van Dam & Vrieling 1994) but the genetic foundation for relaxation of the response is largely unknown. It would be reasonable to expect that selection on the individual would lead to a frequency of undefended

plants such that the fitness of both phenotypes are equal. It may be however, that other factors constrain the maximum relaxation rate and maintain the defended phenotype frequency at levels greater than that at which the fitness of the undefended and defended phenotypes are equal. It seems unlikely that relaxation will be limited by biosynthetic response times as secondary metabolite degradation in leaf tissue can be increased dramatically by excising leaves from induced plants (Daddona *et al.* 1976; Mihaliak *et al.* 1991; Gershenzon 1994), but potential constraints could arise if the induced response forms an intermediate product that is demand limited. The carbon/nutrient balance hypothesis (Bryant *et al.* 1983; Coley *et al.* 1985), and the more general growth differentiation balance hypothesis (Tuomi *et al.* 1990; Herms & Mattson 1992), propose that induction occurs when an environmental factor limits growth by affecting either the ability to acquire carbon or take up nutrients such that resources in excess of growth requirements are then available for the production of defences. Accordingly, as the balance is restored and the resources are required for growth, the induced response declines. Support for these theories is mixed (reviewed Herms & Mattson 1992; Karban & Baldwin 1997, see Chapter 1), and while resource availability has been shown to effect induction in some examples, I know of no study that has manipulated resources post-induction to look at the effects on the rate of relaxation. Another potential constraint on the maximum relaxation rate may arise if early season herbivory is correlated with the risk of attack by late season non-adapted herbivores and there is a defensive inefficiency associated with the delay in mounting an induced response. Under these circumstances rapid relaxation may not be favoured if it increases the risk of damage at some later date. Evidence for such correlation is presently sparse (Karbon & Adler 1996; Karban *et al.* 1999) and more studies are required. In some cases however, this inefficiency may be quite small. For example Zangerl and Berenbaum (1990) found that constitutive and inducible plants transferred to the same site had equal levels of herbivore damage.

While this thesis has identified intermediate frequencies of the undefended phenotype as those most likely to result in the evolutionary stability of induced resistance, induction could still be stable in annual species, and those perennial species with rapid relaxation rates or high turnover of individuals, despite high frequencies of undefended individuals at the beginning of the season. This is likely to be particularly so when there is appreciable within season overlap between insect generations, even if the inducible population is universally undefended. Provided induction is relatively rapid, early season herbivory either by low densities of insects, or confined to early developing plants, could create a phenotype mosaic if individual plants escape herbivory due to chance or late development respectively. In



addition, if the induction level is related to damage intensity (Karban 1983; Neuvonen & Haukioja 1991), random variation in insect feeding will create a quantitative mosaic of states between which insects can discriminate (Stadler & Buser 1984; Reed *et al.* 1989; Dorschner & Kenny 1992; Huang & Renwick 1994). Subsequent herbivores, as they searched for hosts, would then experience a diverse phenotype environment. Again more work is required to understand how early season natural herbivory affects subsequent herbivore risk in inducible systems. Unfortunately the studies that have examined this question have tended to confound induction with insect host choice (*e.g.* Karban 1983; Williams & Myers 1984; Hunter 1987; Leather *et al.* 1987; Roland & Myers 1987; Fritz 1990).

The evolutionary stability of induction under the model assumptions requires the frequency of induced individuals to be maintained at levels such that their fitness is equal to, or lower than that of uninduced individuals. While this seems plausible, we currently have little direct evidence that this occurs in nature. The studies purporting to test the fitness benefits of induction (Table 5.1) have given a somewhat confused picture. Only Baldwin (1998) found that pre-induced plants could have lower fitness than uninduced plants, but only at low insect densities, and this result seems to be attributable to a physiological cost of defence. Consistent with the evolutionary stability of induction, Karban (1993) found no difference in the fitness of pre-induced and uninduced plants despite induction increasing insect mortality. By contrast, the results of Agrawal (1998, 1999) and at one of Baldwin's sites indicate that pre-induction can increase plant fitness in some instances. Interestingly, this suggests that a constitutive defended strategist may be more fit in these situations because pre-induction is analogous to being constitutively defended with the additional burden of any costs that may be involved in the induction process such as leaf removal. This leaves us with the perplexing question of why in these cases is the response inducible when constitutive defence seems to be more effective? Perhaps the fitness benefits vary with herbivore density, as Baldwin's data seem to show. Variation in herbivory between years or within a season may then on average benefit inducible strategists. Another possibility is that the experimental outcome is directed by the pre-selection of treatment frequencies such that the herbivore distribution between treatments differs from that experienced under natural conditions. In the studies of Karban (1993) and Baldwin (1998), the true phenotype frequency was determined by the background states of the surrounding plant population. In Agrawal's experiments (Agrawal 1998, 1999), the choice of treatments set the initial phenotype frequencies. The greatest potential to depart from the natural phenotype frequencies exists in the absence of background host plants. The conflicting results of these fitness studies and the important role of frequency dependent

**Table 5.1. Studies which have attempted to assess the fitness of induced resistance in the presence of herbivores in a field situation.**

Species	<i>Gossypium thurberi</i> (Malvaceae)	<i>Nicotiana attenuata</i> (Solanaceae)	<i>Raphanus sativus</i> L. (Brassicaceae)	<i>Raphanus sativus</i> L. and <i>R. raphanistrum</i> (Brassicaceae)
<b>Pre-induction treatment</b>	Early season inoculation with <i>Bucculatrix thurberiella</i> reduced larval survival	Application of the methyl ester of jasmonic acid increased nicotine pool	Caged <i>Pieris rapae</i> caterpillars increased glucosinolate content and trichome density	Caged <i>Pieris rapae</i> caterpillars increased leaf size, trichome number and trichome density on 3rd leaf
<b>Controls and additional treatments</b>	- Undamaged - Immediate plant density at 2 levels.	- Uninduced - Leaf removal control at 1 site. - Three herbivore regimes: absent, low, and high.	- Undamaged - Leaf damage	- Undamaged - Leaf damage
<b>Method of plant selection</b>	Selected plants within larger population	Selected plants within larger population	Plants transplanted into plowed field	Plants transplanted into plowed field
<b>Plant fitness measures</b>	Survival, growth, growth rate, number of bolls, seeds per boll and mean seed mass	Lifetime viable seed production, capsules per plant and mass per seed	Mortality, total seed mass and early season flowering	Total number of fruits and seeds, and total fruit mass
<b>Effect of pre-induction</b>	- No effect	- With herbivores absent and at one low density site there was a decrease in capsules per plant and lifetime viable seeds per plant - The second low density site had a small increase in capsules per plant and lifetime viable seeds per plant. - At high herbivore densities there was no effect.	- Decreased plant mortality - Increased early season flowering and total seed mass.	- Increased the total number of fruits and seeds, and fruit mass
<b>Comments</b>	- Previous paper describes effects on insect survival		- No control for inducing insects caged to plant for duration of the experiment	- Potentially confounds changes in trichome density with changes in glucosinolate concentration.
<b>Reference</b>	Karban (1993)	Baldwin (1998)	Agrawal (1998)	Agrawal (1999)

selection identified in the present study, indicate that it would be worthwhile to quantify the effects of induction frequency in the field situation.

While a fitness benefit of pre-induction may be used as circumstantial evidence of selection for resistance, it cannot be interpreted as evidence of the superiority of induction over constitutive defence. Similarly, the alternative finding that the uninduced plants are more fit

may indicate that constitutive undefended strategists may be the most successful. Critically, all of the studies to date have lacked a true uninducible control with which to assess the fitness of an undefended strategy. This is a difficult problem to overcome, although the way forward may be to use uninducible mutants in which the induction pathway has been inhibited, such as exist for tobacco (Baldwin 1998). Obviously the study would have to control for differences in fitness between the mutant and the inducible plants in the absence of herbivory. For simulating constitutive defended individuals, the use of chemical inducers (*e.g.* Baldwin 1998) are to be preferred as these do not incur a cost to the plant of tissue removal during the induction process. Some attempt should also be made to control for the effects of experimenter determined phenotype frequencies, particularly as the magnitude of this effect is largely unknown. Two alternative approaches to this problem are given here. The first is to use natural phenotypic variation due to early season herbivory on a largely uninduced population. This could be most easily accomplished by setting out potted plants within a greater population, collecting the early season eggs or larvae, and replacing them on substituted potted plants to avoid confounding insect host choice with induction. The alternative approach is to estimate the effects of induced phenotype frequency by comparing treatment effects at several different background frequencies. Once these effects are estimated however, it would still be necessary to obtain assessments of the natural levels of inducible phenotype variation.

### **CONCLUDING REMARKS**

Herbivore induced changes in plant quality have now been found in over one hundred different plant species (Karban & Baldwin 1997). A key feature to understanding the evolution of induction is to identify how inducibility affects the risk of herbivory, both in terms of its resistance function and through the frequency-dependent effects on insect host choice. Insect host choice by adapted and partially adapted herbivores has been shown to alter key predictions of previous models of induced resistance evolution and can theoretically select for inducibility as an ESS, or result in stable states containing mixtures of plant defensive phenotypes. Unfortunately, field studies to assess the role of host choice in relation to phenotype frequency are lacking. To understand this process we must first determine whether insect pests can select for different plant strategies and whether direction of selection is dependent on the relative importance of adapted and non-adapted herbivores present in the system. Secondly, we must establish whether selection by the herbivore community for particular plant strategies is dependent on the frequency of the various defence phenotypes that may be expressed in the plant population. And finally, we must ascertain whether

variation in the defence phenotype is maintained in inducible populations and how this relates back to the risk of herbivore. This final point has several aspects to it. The first is to understand what within season and between season patterns emerge from previous herbivory and how this relates to herbivory risk. Secondly, we must understand what constraints there may be on relaxing induced response – for example the role of resource limitation as suggested by the C/N balance hypothesis. One final piece of information is to determine whether there is heritable genetic variation in the factors controlling phenotype frequency, particularly with respect to relaxation rates. It should be noted that studies conducted under field conditions are likely to be the most illuminating in these respects, given the complexity of the interactions in this system.

In the light of the diversity of hypotheses invoked to explain the evolution of inducible resistance, and the rather equivocal evidence for all of them, it seems unlikely that any single mechanism will be responsible. However, frequency-dependent herbivory by adapted insects may be an important determinant of the costs associated with expressing induced resistance and joins a growing list of hypotheses that can account for the presence of induction without invoking a physiological cost to resistance.

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## REFERENCES

- Abramson, C. 1994. A Primer of Invertebrate Learning. Washington, DC.: American Psychological Association.
- Adler, F. R. & Karban, R. 1994. Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. Am. Nat., 144, 813-832.
- Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science, 279, 1201-1202.
- Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology, 80, 1713-1723.
- Agrawal, A. A., Strauss, S. Y. & Stout, M. J. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. Evolution, 53, 1093-1104.
- Agren, J. & Schemske, D. W. 1993. The cost of defense against herbivores - an experimental study of trichome production in *Brassica rapa*. Am. Nat., 141, 338-350.
- Anderson, P. & Alborn, H. 1999. Effects on oviposition behaviour and larval development of *Spodoptera littoralis* by herbivore induced changes in cotton plants. Entomol. Exp. Appl., 92, 45-51.
- Anderson, P., Hilker, M. & Lofqvist, J. 1995. Larval diet influence on oviposition behaviour in *Spodoptera littoralis*. Entomol. Exp. Appl., 74, 71-82.
- Astrom, M. & Lundberg, P. 1994. Plant defence and stochastic risk of herbivory. Evol. Ecol., 8, 288-298.
- Bach, C. E. 1980. Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (Fab). Ecology, 61, 1515-1530.

- Baldwin, I. T. 1988. The alkaloidal responses of wild tobacco to real and simulated herbivory. Oecologia, 77, 378-381.
- Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc. Nat. Acad. Sci. (USA), 95, 8113-8118.
- Baldwin, I. T. & Ohnmeiss, T. E. 1994. Co-ordination of photosynthetic and alkaloid responses to damage in uninducible and inducible *Nicotiana sylvestris*. Ecology, 75, 1003-1014.
- Baur, R., Kostal, V. & Stadler, E. 1996. Root damage by conspecific larvae induces preference for oviposition in cabbage root flies. Entomol. Exp. Appl., 80, 224-227.
- Beck, N. G. & Cameron, P. J. 1990. Comparison of Lepidopteran pest populations and their parasitoids in three vegetable Brassicas. Proc. 43rd NZ Weed & Pest Control Confer., 21-25.
- Bergelson, J. & Purrington, C. B. 1996. Surveying patterns in the cost of resistance in plants. Am. Nat., 148, 536-558.
- Bernays, E. A. & Chapman, R. F. 1998. Phenotypic plasticity in numbers of antennal chemoreceptors in a grasshopper - effects of food. J. Comp. Physiol. A Sens. Neural Behav. Physiol., 183, 69-76.
- Bernays, E. A. & Weiss, M. R. 1996. Induced food preferences in caterpillars: The need to identify mechanisms. Entomol. Exp. Appl., 78, 1-8.
- Bernays, E. A., Bright, K., Howard, J. J., Raubenheimer, D. & Champagne, D. 1992. Variety is the spice of life: frequent switching between foods in polyphagous grasshopper *Taeniopoda eques* Burmeister (Orthoptera: Acrididae). Anim. Behav., 44, 721-731.
- Blaakmeer, A., Hagenbeek, D., van Beek, T. A., de Groot, A. E., Schoonhoven, L. M. & van Loon, J. J. A. 1994. Plant-response to eggs vs host marking pheromone as factors inhibiting oviposition by *Pieris brassicae*. J. Chem. Ecol., 20, 1657-1665.
- Bodnaryk, R. P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry, 31, 2671-2677.

- Bodnaryk, R. P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry, 35, 301-305.
- Bowers, M. A. 1993. Influence of herbivorous mammals on an old-field plant community: years 1-4 after disturbance. Oikos, 67, 129-141.
- Bryant, J. P., Chapin, F. S. & Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos, 40, 357-368.
- Bryant, J. P., Chapin, F. S., Reichardt, P. B. & Clausen, T. P. 1987. Response of winter chemical defence in Alaska paper birch and green alder to manipulation of plant carbon/nutrient balance. Oecologia, 72, 510-514.
- Bryant, J. P., Danell, K., Provenza, F., Reichardt, P. B., Clausen, T. A. & Werner, R. A. 1991. Effects of mammal browsing on the chemistry of deciduous woody plants. In: Phytochemical Induction By Herbivores (Ed. by D. W. Tallamy & M. J. Raupp), pp. 135-154. New York: John Wiley and Sons.
- Bryant, J. P., Reichardt, P. B., Clausen, T. P. & Werner, R. A. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. Ecology, 74, 2072-2084.
- Capinera, J. L. 1993. Host plant selection by *Schistocerca americana* (Orthoptera, Acrididae). Envir. Entomol., 22, 127-133.
- Carew, T. J. 1984. An introduction to cellular approaches used in the analysis of habituation and sensitization in *Aplysia*. In: Habituation, Sensitization, and Behavior (Ed. by H. V. S. Peeke & L. Petrinovich), pp. 205-249. Orlando: Academic Press.
- Clark, C. W. & Harvell, C. D. 1992. Inducible defenses and the allocation of resources - a minimal model. Am. Nat., 139, 521-539.
- Coley, P. D., Bryant, J. P. & Chapin, F. S. 1985. Resource availability and plant herbivore defense. Science, 230, 895-899.



- Corbet, S. A. 1985. Insect chemosensory responses: a chemical legacy hypothesis. Ecol. Entomol., 10, 143-153.
- Cottam, D. A. 1985. Frequency-dependent grazing by slugs and grasshoppers. J. Ecol., 73, 925-933.
- Courtney, S. P. & Chen, G. K. 1988. Genetic variation in oviposition behaviour in the mycophagous *Drosophila suboccidentalis* Spcr. Funct. Ecol., 2, 521-528.
- Courtney, S. P. & Kibota, T. T. 1990. Mother doesn't know best: selection of hosts by ovipositing insects. In: Insect-Plant Interactions (Ed. by E. A. Bernays), pp. 161-188. Boca Raton: CRC Press.
- Courtney, S. P., Chen, G. K. & Gardner, A. 1989. A general model for individual host selection. Oikos, 55, 55-65.
- Craig, T. P., Price, P. W. & Itami, J. K. 1986. Resource regulation by a stem galling sawfly on the arroyo willow. Ecology, 67, 419-425.
- Crawley, M. J. 1983. Herbivory: The Dynamics of Animal-Plant Interactions. Oxford: Blackwell Scientific Publications.
- Crawley, M. J. 1985. Reduction of oak fecundity by low-density herbivore populations. Nature, 314, 163-164.
- Crawley, M. J. 1990. The population dynamics of plants. Phil. Trans. Roy. Soc. Lond. B, 330, 125-140.
- Crawley, M. J. 1993. GLIM for Ecologists. Oxford: Blackwell Scientific.
- Cunningham, J. P., Jallow, M. F. A., Wright, D. J. & Zalucki, M. P. 1998. Learning in host selection in *Helicoverpa armigera* (Hubner) (Lepidoptera, Noctuidae). Anim. Behav., 55, 227-234.

- Cyr, H. & Pace, M. L. 1993. Magnitude and pattern of herbivory in aquatic and terrestrial ecosystems. Nature, 361, 148-150.
- Daddona, P. E., Wright, J. L. & Hutchison, C. R. 1976. Alkaloid catabolism and immobilisation in *Catharantus roseus*. Phytochemistry, 15, 941-945.
- De-Jong, R. & Kaiser, L. 1992. Odour preference of a parasitic wasp depends on order of learning. Experientia, 48, 902-904.
- Dempster, J. P. 1992. Evidence of an oviposition-detering pheromone in the orange-tip butterfly, *Anthocharis cardamines* (L). Ecol. Entomol., 17, 83-85.
- Dethier, V. G. 1982. Mechanisms of host-plant recognition. Entomol. Exp. Appl., 31, 49-56.
- Dewick, P. M. 1975. Pterocarpan biosynthesis: chalcone and isoflavone precursors of demethylhomopteroecarpin and maackiain in *Trifolium pratense*. Phytochemistry, 14, 979-982.
- Dicke, M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods. Entomol. Exp. Appl., 91, 131-142.
- Dicke, M. & Sabelis, M. W. 1989. Does it pay to advertise for body-guards? Towards a cost-benefit analysis of induced synomone production. In: Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants (Ed. by H. Lambers, M. L. Cambridge, H. Konings & T. L. Pons), pp. 341-358. The Hague: SPB Academic Publishing.
- Donaldson, J. S. 1993. Mast-seeding in the cycad genus *Encephalartos*: a test of the predator satiation hypothesis. Oecologia, 94, 262-271.
- Dorschner, K. W. & Kenny, S. T. 1992. Occurrence of hop aphid phagostimulants in the honeydew of aphids. Entomol. Exp. Appl., 65, 49-55.
- Edelstein-Keshet, L. & Rausher, M. D. 1989. The effects of inducible plant defenses on herbivore populations. 1. Mobile herbivores in continuous time. Am. Nat., 133, 787-810.

- Edwards, P. J., Wratten, S. D. & Cox, H. 1985. Wound-induced changes in the acceptability of tomato to larvae of *Spodoptera littoralis*: a laboratory bioassay. Ecol. Entomol., 10, 155-158.
- Ehrlich, P. R. & Raven, P. H. 1964. Butterflies and plants: a study in coevolution. Evolution, 18, 586-608.
- English-Loeb, G. M. & Karban, R. 1991. Consequences of mite feeding injury to beans on the fecundity and survivorship of the two spotted mite (Acari: Tetranychidae). Exp. & Appl. Acarol., 11, 125-136.
- Euler, M. & Baldwin, I. T. 1996. The chemistry of defence and apparency in the corollas of *Nicotiana attenuata*. Oecologia, 107, 102-112.
- Feeny, P. 1975. Biochemical coevolution between plants and their insect herbivores. In: Coevolution of Animals and Plants (Ed. by L. E. Gilbert & P. H. Raven), pp. 3-19. Austin: University of Texas Press.
- Feeny, P. 1976. Plant apparency and chemical defence. Recent Adv. Phytochem., 10, 1-40.
- Fienberg, S. E. 1980. The Analysis of Cross-Classified Categorical Data. 2nd edn. Cambridge: MIT Press.
- Fineblum, W. L. & Rausher, M. D. 1995. Trade-off between resistance and tolerance to herbivore damage in a morning glory. Nature, 377, 517-520.
- Firempong, S. & Zalucki, M. P. 1991. Host plant selection by *Helicoverpa armigera* (Lepidoptera, Noctuidae) - the role of some herbivore attributes. Aust. J. Zool., 39, 343-350.
- Fitt, G. P. 1986. The influence of a shortage of hosts on the specificity of oviposition behaviour in species of *Dacus* (Diptera, Tephritidae). Physiol. Entomol., 11, 133-143.
- Fowler, S. V. & Lawton, J. H. 1985. Rapidly induced defences and talking trees: the devil's advocate position. Am. Nat., 126, 181-195.
- Frank, S. A. 1993. A model of inducible defense. Evolution, 47, 325-327.

Fritz, R. S. 1990. Variable competition between insect herbivores on genetically variable host plants. Ecology, 71, 1208-1211.

Gershenzon, J. 1994. The cost of plant chemical defense against herbivory: a biochemical perspective. In: Insect-Plant Interactions (Ed. by E. Bernays), pp. 105-173. Boca Raton: CRC Press.

Giamoustaris, A. & Mither, R. 1995. The effect of modifying glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. Annals Appl. Biol., 126, 347-363.

Gianoli, E. & Niemeyer, H. M. 1997. Lack of costs of herbivory-induced defences in a wild wheat - integration of physiological and ecological approaches. Oikos, 80, 269-275.

Groeters, F. R., Tabashnik, B. E., Finson, N. & Johnson, M. W. 1992. Oviposition preference of the diamondback moth (*Plutella xylostella*) unaffected by the presence of conspecific eggs or *Bacillus thuringiensis*. J. Chem. Ecol., 18, 2353-2362.

Gulmon, S. L. & Mooney, H. A. 1986. Costs of defence and their effects on plant productivity. In: On the Economy of Plant Form and Function (Ed. by T. J. Givnish), pp. 681-698. Cambridge: Cambridge University Press.

Gupta, P. D. & Thorsteinson, A. J. 1960. Food plant relationships of the diamondback moth (*Plutella maculipennis* (Curt.)) 2. sensory regulation of oviposition of the adult female. Entomol. Exp. Appl., 3, 305-314.

Hansson, B. S., Larsson, M. C. & Leal, W. S. 1999. Green leaf volatile-detecting olfactory receptor neurons display very high sensitivity and specificity in a scarab beetle. Physiol. Entomol., 24, 121-126.

Harcourt, D. G. 1986. Population dynamics of the diamondback moth in southern Ontario. In: Diamondback Moth Management: Proceedings of the First International Workshop (Ed. by N. S. Talekar), pp. 3-15. Shanhu: Asian Vegetable Research and Development Centre.

- Haukioja, E. & Neuvonen, S. 1985. Induced long-term resistance of birch foliage against defoliators: defensive or incidental? Ecology, 66, 1303-1308.
- Henderson, C. B. 1990. The influence of seed apparency, nutrient content and chemical defences on dietary preference in *Dipodomys ordii*. Oecologia, 82, 333-341.
- Hermes, D. A. & Mattson, W. J. 1992. The dilemma of plants - to grow or defend. Quart. Rev. Biol., 67, 283-335.
- Hillyer, R. J. & Thorsteinson, J. 1969. The influence of the host plant or males on ovarian development or oviposition in the diamondback moth *Plutella maculipennis* (Curt.). Can. J. Zool., 47, 805-816.
- Honkanen, T., Haukioja, E. & Kitunen, V. 1999. Responses of *Pinus sylvestris* branches to simulated herbivory are modified by tree sink/source dynamics and by external resources. Funct. Ecol., 13, 126-140.
- Hopkins, A. D. 1917. A discussion of C.G Hewitt's paper on "Insect Behavior". J. Econ. Entomol., 10, 92.
- Horton, D. R. & Krysan, J. L. 1991. Host acceptance behavior of pear psylla (Homoptera, Psyllidae) affected by plant-species, host deprivation, habituation, and eggload. Ann. Ent. Soc. America, 84, 612-627.
- Huang, X. P. & Renwick, J. A. A. 1994. Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi oleracea*. J. Chem. Ecol., 20, 1025-1037.
- Hunt, D. W. A. & Whitfield, G. 1996. Potato trap crops for control of Colorado potato beetle (Coleoptera: Chrysomelidae) in tomatoes. Can. Ent., 128, 407-412.
- Hunter, M. D. 1987. Opposing effects of spring defoliation on late season oak caterpillars. Ecol. Entomol., 12, 373-382.
- Islam, Z. & Crawley, M. J. 1983. Compensation and regrowth in ragwort (*Senecio jacobaea*) attacked by cinnabar moth (*Tyria jacobaeae*). J. Ecol., 71, 829-843.

- Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. Theor. Pop. Biol., 14, 350-356.
- Jaenike, J. 1983. Induction of host preference in *Drosophila melanogaster*. Oecologia, 58, 320-325.
- Jaenike, J. 1990. Host Specialization in Phytophagous Insects. Ann. Rev. Ecol. Syst., 21, 243-273.
- Jaenike, J. & Papaj, D. 1992. Behavioural plasticity and patterns of host use by insects. In: Insect Chemical Ecology (Ed. by B. Roitberg & M. Isman), pp. 245-264. New York: Chapman and Hall.
- de Jong, G. 1979. The influence of the distribution of juveniles over patches of food on the dynamics of a population. Netherl. J. Zool., 29, 33-51.
- Karban, R. 1983. Induced responses of cherry trees to periodical cicada oviposition. Oecologia, 59, 226-231.
- Karban, R. 1993. Costs and benefits of induced resistance and plant density for a native shrub, *Gossypium thurberi*. Ecology, 74, 9-19.
- Karban, R. 1997. Neighbourhood affects a plants risk of herbivory and subsequent success. Ecol. Entomol., 22, 433-439.
- Karban, R. & Adler, F. R. 1996. Induced resistance to herbivores and the information content of early season attack. Oecologia, 107, 379-385.
- Karban, R. & Baldwin, I. T. 1997. Induced Responses to Herbivory. Chicago: University of Chicago Press.
- Karban, R. & Myers, J. H. 1989. Induced plant responses to herbivory. Ann. Rev. Ecol. Syst., 20, 331-348.

- Karban, R., Agrawal, A. A. & Mangel, M. 1997. The benefits of induced defenses against herbivores. Ecology, 78, 1351-1355.
- Karban, R., Agrawal, A. A., Thaler, J. S. & Adler, L. 1999. Induced plant responses and information content about risk of herbivory. Tr. Ecol. Evo., 14, 443-447.
- Karieva, P. 1982. Experimental and mathematical analyses of herbivore movement: quantifying the influence of plant spacing and quality on foraging discrimination. Ecol. Monog., 52, 261-282.
- Kielkiewicz, M. 1988. Susceptibility of previously damaged strawberry plants to mite attack. Entomol. Exp. Appl., 47, 201-203.
- Kumar, H. 1992. Inhibition of ovipositional responses of *Chilo partellus* (Lepidoptera: Pyralidae) by the trichomes on the lower leaf surface of a maize cultivar. J. Econ. Entomol., 85, 1736-1739.
- Lamb, R. J. 1989. Entomology of oilseed *Brassica* crops. Ann. Rev. Entomol., 34, 211-229.
- Landolt, P. J. 1993. Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. Entomol. Exp. Appl., 67, 79-85.
- Landolt, P. J. & Molina, O. 1996. Host-finding by cabbage looper moths (Lepidoptera: Noctuidae): learning of host odour upon contact with host foliage. J. Insect Behav., 9, 899-908.
- Lawrence, R. K., Mattson, W. J. & Haack, R. A. 1997. White spruce and the spruce budworm - defining the phenological window of susceptibility. Can. Ent., 129, 291-318.
- Leather, S. R. 1993. Early season defoliation of bird cherry influences autumn colonisation by the bird cherry aphid, *Rhopalosiphum padi*. Oikos, 66, 43-47.
- Leather, S. R. & Walsh, P. J. 1993. Sublethal plant defenses - the paradox remains. Oecologia, 93, 153-155.

- Leather, S. R., Watt, A. D. & Forrest, G. I. 1987. Insect-induced chemical changes in young lodgepole pine (*Pinus contorta*): the effect of previous defoliation on oviposition, growth and survival of the pine beauty moth, *Panolis flammea*. Ecol. Entomol., 12, 275-281.
- Lee, H. J. & Tseng, Y. C. 1993. Induction of host plant choice for feeding and oviposition in diamondback moth larvae (*Plutella xylostella*). Bull. Inst. Zool. Acad. Sinica, 32, 148-152.
- Levins, R. 1968. Evolution in Changing Environments. Princeton, NJ: Princeton University Press.
- Lindstrom, M., Norin, T. & Sjodin, K. 1990. Plant constituents as signals for aggregation and attack of pest insects. Pure Appl. Chem., 62, 1329-1332.
- Lively, C. M. 1986. Canalization versus developmental conversion in a spatially variable environment. Am. Nat., 128, 561-572.
- Lloyd, D. G. 1984. Variation strategies of plants in heterogeneous environments. Biol. J. Linn. Soc., 21, 357-385.
- Loomis, W. E. 1953. Growth and differentiation - an introduction and summary. In: Growth and Differentiation in Plants (Ed. by W. E. Loomis), pp. 1-17. Ames: Iowa State University Press.
- Mackay, D. A. 1985. Prealighting search behavior and host plant selection by ovipositing *Euphydryas editha* butterflies. Ecology, 66, 142-151.
- Maddox, G. D. & Root, R. B. 1987. Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. Oecologia, 72, 8-14.
- Mangel, M. 1993. Motivation, learning, and motivated learning. In: Insect Learning (Ed. by D. Papaj & A. Lewis), pp. 158-173. New York: Chapman and Hall.
- Marquis, R. J. 1992. A bite is a bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). Ecology, 73, 143-152.



- Marquis, R. J. 1996. Plant architecture, sectoriality and plant tolerance to herbivores. Vegetatio, 127, 85-97.
- Mathsoft Inc. 1995. S-Plus: Version 3.3. .
- Mathsoft Inc. 1998. S-Plus: Version 4.5. .
- Mauricio, R. 1998. Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. Am. Nat., 151, 20-28.
- Mauricio, R. & Rausher, M. D. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. Evolution, 51, 1435-1444.
- Mauricio, R., Rausher, M. D. & Burdick, D. S. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? Ecology, 78, 1301-1311.
- Maurico, R., Bowers, M. D. & Bazzaz, F. A. 1993. Pattern of leaf damaged affects fitness of the annual plant *Raphanus sativus* (Brassicaceae). Ecology, 74, 2066-2071.
- May, R. M. 1976. Simple mathematical models with very complicated dynamics. Nature, 261, 459-467.
- May, R. M. & Oster, G. F. 1976. Bifurcations and dynamic complexity in simple ecological models. Am. Nat., 110, 573-599.
- Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. Oikos, 79, 417-428.
- Maynard-Smith, J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press.
- McCullagh, P. & Nelder, J. A. 1989. Generalized Linear Models. 2nd edn. London: Chapman and Hall.

- Mead, R. 1988. The Design of Experiments. Cambridge: Cambridge University Press.
- Messina, F. J., Kemp, J. L. & Dickinson, J. A. 1992. Plasticity in the egg-spacing behavior of a seed beetle: effects of host deprivation and seed patchiness (Coleoptera: Bruchidae). J. Insect Behav., 5, 609-621.
- Messina, F. J., Jones, T. A. & Nielson, D. C. 1993. Performance of the Russian wheat aphid (Homoptera, Aphididae) on perennial range grasses - effects of previous defoliation. Envir. Entomol., 22, 1349-1354.
- Metcalf, R. L. & Lampman, R. L. 1989. The chemical ecology of Diabroticites and Cucurbitaceae. Experientia, 45, 240-247.
- Mihaliak, C. A., Gershenzon, J. & Croteau, R. 1991. Lack of rapid monoterpene turnover in rooted plants: implications for theories of plant chemical defence. Oecologia, 87, 373-376.
- Miller, J. R. & Strickler, K. L. 1984. Finding and accepting host plants. In: Chemical Ecology of Insects (Ed. by W. J. Bell & R. T. Carde), pp. 127-157. London: Chapman and Hall.
- Minkenberg, O. P. J. M., Tatar, M. & Rosenheim, J. A. 1992. Egg load as a major source of variability in insect foraging and oviposition behavior. Oikos, 65, 134-142.
- Mopper, S., Whitham, T. G. & Price, P. W. 1990. Plant phenotype and interspecific competition between insects determine sawfly performance and density. Ecology, 71, 2135-2144.
- Nault, L. R. & Steyer 1972. Effects of sinigrin on host selection by aphids. Entomol. Exp. Appl., 15, 423-433.
- Neuvonen, S. & Haukioja, E. 1991. The effects of inducible resistance in host foliage on birch-feeding herbivores. In: Phytochemical Induction By Herbivores (Ed. by D. W. Tallamy & M. J. Raupp), pp. 277-291. New York: John Wiley and Sons.
- Nichols-Orians, C. M. 1991. The effects of light on foliar chemistry, growth and susceptibility of seedlings of a canopy tree to an attine ant. Oecologia, 86, 552-560.

- Odendaal, F. J. & Rausher, M. D. 1990. Egg load influences search intensity, host selectivity and clutch size in *Battus philenor* butterflies. J. Insect Behav., 3, 183-193.
- Pacala, S. W. & Crawley, M. J. 1992. Herbivores and plant diversity. Am. Nat., 140, 243-260.
- Padilla, D. K. & Adolph, S. C. 1996. Plastic inducible morphologies are not always adaptive - the importance of time delays in a stochastic environment. Evol. Ecol., 10, 105-117.
- Papaj, D. R. & Prokopy, R. J. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. Ann. Rev. Entomol., 34, 315-350.
- Papaj, D. R. & Rausher, M. D. 1983. Individual variation in host location by phytophagous insects. In: Herbivorous Insects: Host Seeking Behaviour and Mechanisms (Ed. by S. Ahmad), pp. 77-124. New York: Academic Press.
- Parker, M. A. & Root, R. B. 1981. Insect herbivores limit habitat distribution of a native composite, *Machaeranthera canescens*. Ecology, 62, 1390-1392.
- Parmesan, C., Singer, M. C. & Harris, I. 1995. Absence of adaptive learning from the oviposition foraging behaviour of a checkerspot butterfly. Anim. Behav., 50, 161-175.
- Petrinovich, L. 1984. A two-factor dual-process theory of habituation and sensitization. In: Habituation, Sensitization, and Behavior (Ed. by H. Peeke & L. Petrinovich), pp. 17-55. Orlando: Academic Press.
- Philippi, T. & Seger, J. 1989. Hedging one's evolutionary bets, revisited. Tr. Ecol. Evo., 4, 41-44.
- Pivnick, K. A., Jarvis, B. J., Gillott, C., Slater, G. P. & Underhill, E. A. 1990a. Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth (Lepidoptera: Plutellidae). Envir. Entomol., 19, 587-593.
- Pivnick, K. A., Jarvis, B. J., Gillott, C., Slater, G. P. & Underhill, E. W. 1990b. Attraction of the diamondback moth (Lepidoptera: Plutellidae) to volatiles of oriental mustard: the influence of age, sex and prior exposure to mates and host plants. Envir. Entomol., 19, 704-709.

- Pivnick, K. A., Jarvis, B. J. & Slater, G. P. 1994. Identification of olfactory cues used in host-plant finding by diamondback moth, *Plutella xylostella* (Lepidoptera, Plutellidae). J. Chem. Ecol., 20, 1407-1427.
- Potter, D. A. & Redmond, C. T. 1989. Early spring defoliation, spring leaf flush, and leafminer outbreaks on American holly. Oecologia, 81, 192-197.
- Prokopy, R. J., Cooley, S. S. & Papaj, D. R. 1993. How well can relative specialist *Rhagoletis* flies learn to discriminate fruit for oviposition? J. Insect Behav., 6, 167-176.
- Prokopy, R. J., Bergweiler, C., Galarza, L. & Schwerin, J. 1994a. Prior experience affects the visual ability of *Rhagoletis pomonella* flies (Diptera: Tephritidae) to find host fruit. J. Insect Behav., 7, 663-677.
- Prokopy, R. J., Roitberg, B. D. & Vargas, R. I. 1994b. Effects of egg load on finding and acceptance of host fruit in *Ceratitis capitata* flies. Physiol. Entomol., 19, 124-132.
- Pullin, A. S. 1987. Changes in leaf quality following clipping and regrowth in *Urtica dioica* and consequences for a specialist insect herbivore, *Aglas urticae*. Oikos, 49, 39-45.
- Quiring, D. T. & Butterworth, E. W. 1994. Genotype and environment interact to influence acceptability and suitability of white spruce for a specialist herbivore, *Zeraphera canadensis*. Ecol. Entomol., 19, 230-238.
- Raupp, M. J. & Denno, R. F. 1984. The suitability of damaged willow leaves as food for the leaf beetle, *Palgiodera versicola*. Ecol. Entomol., 9, 443-448.
- Rausher, M. D. 1983a. Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. Ecology, 64, 1028-1034.
- Rausher, M. D. 1983b. Conditioning and genetic variation as causes of individual variation in the oviposition behaviour of the tortoise beetle *Deloyala guttata*. Anim. Behav., 31, 743-747.

- Rausher, M. D. & Odendaal, F. J. 1987. Switching and the pattern of host use by *Battus philenor* butterflies. Ecology, 68, 869-877.
- Reed, D. W., Pivnick, K. A. & Underhill, E. W. 1989. Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in 3 species of Brassicaceae. Entomol. Exp. Appl., 53, 277-286.
- Renwick, J. A. A. & Chew, F. S. 1994. Oviposition behaviour in Lepidoptera. Ann. Rev. Entomol., 39, 377-400.
- Riggin-Bucci, T. M. & Gould, F. 1996. Effects of surfactants, *Bacillus thuringiensis* formulations and plant damage on oviposition by diamondback moth. Ecol. Entomol., 89, 891-897.
- Robertson, P. L. 1939. Diamondback moth investigation in New Zealand. N.Z. J. Sci. Tech., 20, 330-363.
- Rogers, S. M. & Simpson, S. J. 1997. Experience-dependent changes in the number of chemosensory sensilla on the mouthparts and antennae of *Locusta migratoria*. J. Exp. Biol., 200, 2313-2321.
- Roitberg, B. D. & Prokopy, R. J. 1987. Insects that mark hosts plants. Biosci., 37, 400-406.
- Rojas, J. C. & Wyatt, T. D. 1999. The role of pre-imaginal and post-imaginal experience in the host-finding and oviposition behavior of the cabbage moth. Physiol. Entomol., 24, 83-89.
- Roland, J. & Myers, J. H. 1987. Improved insect performance from host-plant defoliation: winter moth on oak and apple. Ecol. Entomol., 12, 409-414.
- Root, R. B. 1996. Herbivore pressure on goldenrods (*Solidago altissima*): its variation and cumulative effects. Ecology, 77, 1074-1087.
- Rosenheim, J. A. & Rosen, D. 1991. Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis* - distinguishing the influences of egg load and experience. J. Anim. Ecol., 60, 873-893.

- Schindek, R. & Hilker, M. 1996. Influence of larvae of *Gastrophysa viridula* on the distribution of conspecific adults in the field. Ecol. Entomol., 21, 370-376.
- Schoener, T. W. 1971. Theory of feeding strategies. Ann. Rev. Entomol., 2, 369-404.
- Scriber, J. M. 1978. Cyanogenic glycosides in *Lotus corniculatus*: their effect upon growth, energy budget, and nitrogen utilization of the southern armyworm *Spodoptera eridania*. Oecologia, 34, 143-155.
- Scriber, J. M. 1993. Absence of behavioural induction in oviposition preference of *Papilio glaucus* (Lepidoptera: Papilionidae). Great Lakes Entomol., 26, 81-95.
- Scriber, J. M. & Slansky, F. 1981. The Nutritional Ecology of Immature Insects. Ann. Rev. Entomol., 26, 183-211.
- Simms, E. L. 1992. Costs of plant resistance to herbivores. In: Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics (Ed. by R. S. Fritz & E. L. Simms), pp. 392-425. Chicago: University of Chicago Press.
- Simms, E. L. & Rausher, M. D. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. Evolution, 43, 573.
- Singer, M. C. 1982. Quantification of host preference by manipulation of oviposition behaviour in the butterfly *Euphydryas editha*. Oecologia, 52, 224-229.
- Singer, M. C. 1983. Determinants of multiple host use by a phytophagous insect population. Evolution, 37, 389-403.
- Singer, M. 1986. The definition and measurement of oviposition preference in plant-feeding insects. In: Insect-Plant Interactions (Ed. by J. Miller & T. Miller), pp. 65-94. New York: Springer-Verlag.

- Singer, M. C., Vasco, D., Parmesan, C., Thomas, C. D. & Ng, D. 1992. Distinguishing between preference and motivation in food choice - an example from insect oviposition. Anim. Behav., 44, 463-471.
- Smithson, A. & Macnair, M. R. 1997. Density-dependent and frequency-dependent selection by bumblebees *Bombus terrestris* (L) (Hymenoptera, Apidae). Biol. J. Linn. Soc., 60, 401-417.
- Solarz, S. L. & Newman, R. M. 1996. Oviposition specificity and behaviour of the watermilfoil specialist *Euhrychiopsis lecontei*. Oecologia, 106, 337-344.
- Spencer, J. L. 1996. Waxes enhance *Plutella xylostella* oviposition in response to sinigrin and cabbage homogenates. Entomol. Exp. Appl., 81, 165-173.
- Srinivasan, K., Moorthy, P. N. K. & Raviprasad, T. N. 1994. African marigold as a trap crop for the management of the fruit borer *Helicoverpa armigera* on tomato. International Journal of Pest Management, 40, 56-63.
- Stadler, E. & Buser, H. R. 1984. Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. Experientia, 40, 1157-1159.
- Stanek, E. J., Diehl, S. R., Dgetluck, N., Stokes, M. E. & Prokopy, R. J. 1987. Statistical methods for analysing discrete responses of insects tested repeatedly. Envir. Entomol., 16, 319-326.
- Stanton, M. L. 1984. Short-term learning and the searching accuracy of egg-laying butterflies. Anim. Behav., 32, 33-40.
- Stephens, D. W. 1993. Learning and behavioral ecology: incomplete information and environmental predictability. In: Insect Learning (Ed. by D. R. Papaj & A. C. Lewis), pp. 195-218. New York: Chapman and Hall.
- Stowe, K. A. 1998. Experimental evolution of resistance in *Brassica rapa* - correlated response of tolerance in lines selected for glucosinolate content. Evolution, 52, 703-712.
- Strang, G. 1991. Calculus. Wellesley MA: Wellesley-Cambridge Press.

- Strauss, S. Y. & Agrawaal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. Tr. Ecol. Evo., 14, 179-185.
- Szentesi, A. & Jermy, T. 1990. The role of experience in host plant choice by phytophagous insects. In: Insect-Plant Interactions (Ed. by E. A. Bernays), pp. 39-74. Boca Raton: CRC Press.
- Talekar, N. S. & Shelton, A. M. 1993. Biology, ecology, and management of the diamondback moth. Ann. Rev. Entomol., 38, 275-301.
- Tallamy, D. W. 1985. Squash beetle feeding behaviour: an adaptation against induced cucurbit defences. Ecology, 66, 1574-1579.
- Tallamy, D. W. & McCloud, E. S. 1991. Squash beetles, cucumber beetles, and inducible cucurbit responses. In: Phytochemical Induction By Herbivores (Ed. by D. W. Tallamy & M. J. Raupp), pp. 155-181. New York: John Wiley and Sons.
- Thaler, J. S. 1999. Jasmonate-inducible plant defenses cause increased parasitism of herbivores. Nature, 399, 686-688.
- Thomas, C. D. & Singer, M. C. 1987. Variation in host preference affects movement patterns within a butterfly population. Ecology, 68, 1262-1297.
- Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. Exp. Appl., 47, 3-14.
- Thompson, J. N. & Pellmyr, O. 1991. Evolution of oviposition behaviour and host preference in Lepidoptera. Ann. Rev. Entomol., 36, 65-89.
- Till-Bottraud, I. & Grouyon, P. 1992. Intra- versus interplant batesian mimicry? A model of cyanogenesis and herbivory in clonal plants. Am. Nat., 139, 509-520.
- Tuomi, J., Vuorisalo, T., Jormalainen, V., Jussila, I. & Niemela, P. 1989. Delayed budbreak: a defensive response of mountain birch to early season defoliation. Oikos, 54, 87-91.



- Tuomi, J., Niemela, P. & Siren, S. 1990. The Panglossian paradigm and delayed inducible accumulation of foliar phenolics in mountain birch. Oikos, 59, 399-410.
- Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Rose, U. S. R., Lewis, W. J. & Tumlinson, J. H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Nat. Acad. Sci. (USA), 92, 4169-4174.
- Uematsu, H. & Sakanoshita, A. 1993. Micro-distribution of eggs on diamondback moth, *Plutella xylostella*, on intact and injured cabbage plantlets. Jap. J. Appl. Entomol. Zool., 37, 1-3.
- Underwood, N. 1999. The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. Am. Nat., 153, 282-294.
- van Dam, N. M. & Vrieling, K. 1994. Genetic variation in constitutive and inducible pyrrolizidine alkaloid levels in *Cynoglossum officinale* L. Oecologia, 99, 374-378.
- van Emden, H. F., Sponagl, B., Baker, T., Ganguly, S. & Douloumpaka, S. 1996. Hopkins host selection principle, another nail in its coffin. Physiol. Entomol., 21, 325-328.
- Wallner, W. E. & Walton, G. S. 1979. Host defoliation: a possible determinant of gypsy moth population quality. Ann. Ent. Soc. America, 72, 62-67.
- Wasserman, S. S. & Futuyma, D. J. 1981. Evolution of host plant utilisation in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus*, Fabricius (Coleoptera: Bruchidae). Evolution, 35, 605-617.
- Webb, S. W. & Moran, V. C. 1978. The influence of the host plant on the population dynamics of *Acizzia russellae* (Homoptera: Psyllidae). Ecol. Entomol., 3, 313-321.
- Wiklund, C. 1981. Generalist versus specialist behaviour in *Papilio machaon* (Lepidoptera) and functional aspects on the hierarchy of oviposition preferences. Oikos, 36, 163-170.
- Wilcox, A. & Crawley, M. J. 1988. The effects of host plant defoliation and fertiliser application on larval growth and oviposition behaviour in cinnabar moth. Oecologia, 76, 283-287.

- Williams, K. S. & Myers, J. H. 1984. Previous herbivore attack of red alder may improve food quality for fall webworm larvae. *Oecologia*, 63, 166-170.
- Wise, M. J. & Sacchi, C. F. 1996. Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*. *Oecologia*, 108, 328-337.
- Wold, E. N. & Marquis, R. J. 1997. Induced defense in white oak: effects on herbivores and consequences for the plant. *Ecology*, 78, 1356-1369.
- Young, T. P. & Okello, B. D. 1998. Relaxation of an induced defence after exclusion of herbivores: spines on *Acacia drepanolobium*. *Oecologia*, 115, 508-513.
- Zangerl, A. R. & Berenbaum, M. R. 1990. Furanocoumarin induction in wild parsnip: genetics and population variation. *Ecology*, 71, 1933-1940.
- Zar, J. H. 1984. *Biosatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall International.

## APPENDIX A

### MATHEMATICAL PROOFS

#### *A.1 EVOLUTIONARY STABILITY WHEN $W(A', A) = W(A, A)$ .*

This analysis is restricted to considering invasion of either a constitutive undefended or inducible population by a single constitutive defended strategist. When the fitness of both invading and common strategies are equal, the requirement for evolutionary stability has been previously stated as:

$$W(A', A) = W(A, A) \text{ and for small } \varepsilon, \quad W(A'_\varepsilon, A_\varepsilon) < W(A_\varepsilon, A_\varepsilon) \quad (4.20b)$$

First we must consider the effect of an increase in the frequency of a single constitutive defended strategist in an infinite population to  $\varepsilon$ . An increase in the frequency of the defended strategy will arise through the displacement of new individuals of either common strategy from vacated sites. Hence the frequency of the defended phenotype will increase because the inducible and constitutive undefended strategies enter the population undefended. We are then required to know what the effect of a small increase in the frequency of the defended phenotype is on the respective fitnesses of the strategies. We can proceed in the following manner. The constitutive undefended equation (equation 4.29) and the inducible stability equation (equation 4.30) can both be written in the form:

$$\frac{\hat{m}_{def} - \hat{m}_{undef}}{\hat{g}_{undef}} > \frac{1 - e^{-2\theta_s F_s \tau}}{1 - e^{-2\theta_m F_m}} \quad (i)$$

When  $\tau = 1$ , this more general form yields the equivalent of equation (4.22). Let  $y$  = LHS of equation (i). Using equation (4.8),  $y$  can be written as:

$$\frac{\hat{M}}{\hat{G}} \left( \frac{f_{undef,t} E_{def,t}}{f_{def,t}} - E_{undef,t} \right) \quad (ii)$$

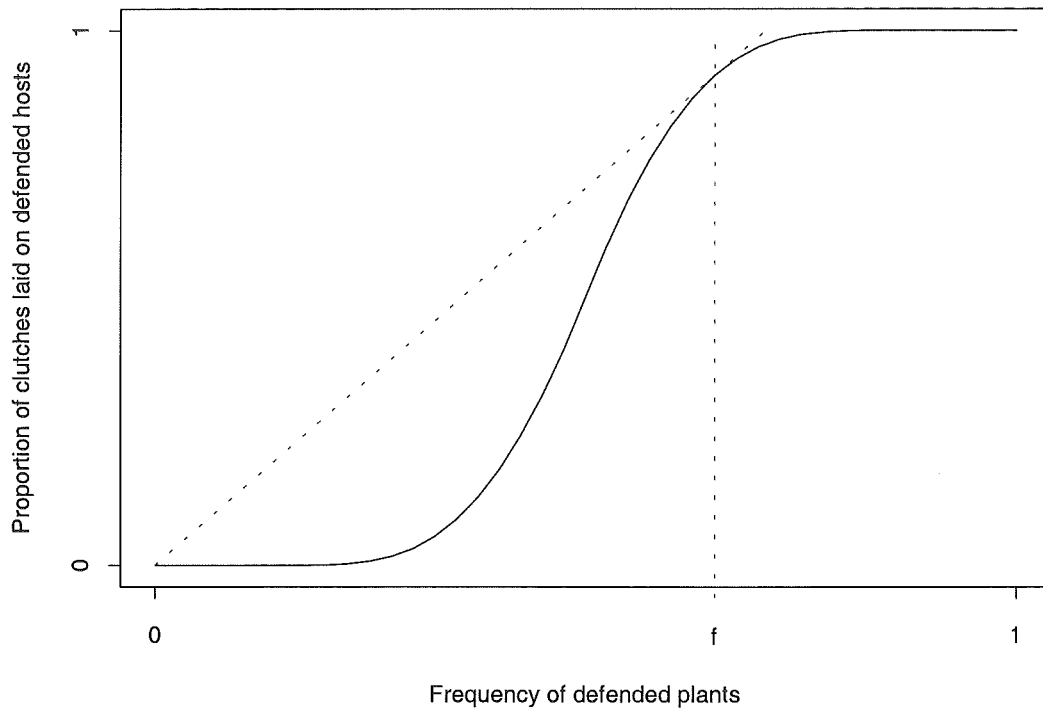
where  $\hat{M}$  and  $\hat{G}$  are the equilibrium global densities of adapted and non-adapted herbivores respectively. Making use of the property that  $f_{undef,t} = 1 - f_{def,t}$  and that  $E_{undef,t} = 1 - E_{def,t}$ , the derivative with respect to  $f_{def,t}$  of equation (ii) is:

$$\frac{d}{df_{def,t}} = \frac{\hat{M}}{\hat{G}} \left( \frac{1}{f_{def,t}} \left( \frac{dE_{def,t}}{df_{def,t}} - \frac{E_{def,t}}{f_{def,t}} \right) \right) \quad (\text{iii})$$

The common strategy induction or constitutive undefended will be stable provided  $d/df_{def,t} \geq 0$  for any small increase in the frequency of the defended phenotype. Hence for non-zero  $\hat{M}$  we require:

$$\frac{dE_{def,t}}{df_{def,t}} - \frac{E_{def,t}}{f_{def,t}} \geq 0 \quad (\text{iv})$$

This condition holds provided the frequency of the defended phenotype is sufficiently small (Figure A.1). The exact limits depend on the particular acceptance probability curve.



**Figure A.1.** The proportion of clutches laid on the defended phenotype ( $E_{def}$ ) as a function of the defended phenotype frequency. " $f$ " denotes the frequency at which  $dE_{def,t} / df_{def,t} = E_{def,t} / f_{def,t}$  and is obtained by the value of  $f_{def}$  at which the tangent to the acceptance probability curve goes through the origin. At the stability boundary, the inducible or constitutive undefended strategies are resistant to invasion by the constitutive defended strategy for all values of  $f_{def,t} \leq "f"$ .

## A.2 INDUCTION CAN BE AN ESS FOR ALL NON-ADAPTED HERBIVORE DENSITIES

Let the general evolutionary stability conditions for an inducible population in the presence of non-adapted and adapted or partially adapted herbivores be given by:

$$\begin{aligned} & \exp(-0.5(\hat{m}_{undef}(1 - e^{-2\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_s F_s}))) < \\ & (1 - f_{undef}) \exp(-0.5\hat{m}_{def}(1 - e^{-2Q\theta_m F_m})) + \\ & f_{undef} \exp(-0.5(\hat{m}_{undef}(1 - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + g_{undef}(1 - e^{-2\theta_s F_s}))) \end{aligned} \quad (v) = (4.35)$$

against constitutive undefended strategists and

$$\begin{aligned} & \exp(-0.5\hat{m}_{def}(1 - e^{-2Q\theta_m F_m})) < \\ & \exp(-0.5(\hat{m}_{undef}(1 - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_s F_s}))) \end{aligned} \quad (vi) = (4.36)$$

against constitutive undefended strategists. For adapted insects,  $Q = 1$  yielding equations 4.29 and 4.30 respectively after some manipulation. For partially adapted insects,  $Q$  is constrained such that  $0 < Q < 1$ . Manipulation of equations (v) and (vi) yields the stability conditions in terms of the difference between the density of adapted or partially adapted insects on defended plants and undefended plants ( $\hat{m}_{def} - \hat{m}_{undef}$ ) or:

$$\hat{m}_{def} - \hat{m}_{undef} > \hat{g}_{undef}(1 - e^{-2\theta_s F_s}) + \hat{m}_{def}e^{-2Q\theta_m F_m} - \hat{m}_{undef}e^{-2(Q+\tau(1-Q))\theta_m F_m} \quad (vii)$$

and

$$\begin{aligned} & \hat{m}_{def} - \hat{m}_{undef} < \hat{m}_{def}e^{-2Q\theta_m F_m} - \hat{m}_{undef}e^{-2\theta_m F_m} - \\ & 2 \ln \left( \frac{\exp(-0.5\hat{g}_{undef}(1 - e^{-2\theta_s F_s})) - f_{undef} \exp(-0.5(\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_s F_s})))}{1 - f_{undef}} \right) \end{aligned} \quad (viii)$$

Combining equations (vii) and (viii) gives the equivalent to equation 4.31 for adapted insects and 4.37 for partially adapted insects. Provided equation (viii) is defined (when it is not it is no longer limiting), there will be a value of  $\hat{m}_{def} - \hat{m}_{undef}$  such that induction is an ESS for all positive  $\hat{g}_{undef}$  if equation (viii) is greater than or equal to equation (vii). Let equation (viii) = LHS and equation (vii) = RHS. We require LHS > RHS. Subtracting  $\hat{m}_{def} e^{-2Q\theta_m F_m} - \hat{m}_{undef} e^{-2\theta_m F_m}$  from both sides and multiplying by -0.5 gives:

$$\ln \left( \frac{\exp(-0.5\hat{g}_{undef}(1 - e^{-2\theta_s F_s})) - f_{undef} \exp(-0.5(\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}) + \hat{g}_{undef}(1 - e^{-2\theta_s F_s \tau})))}{1 - f_{undef}} \right) < \quad (ix)$$

$$-0.5(\hat{g}_{undef}(1 - e^{-2\theta_s F_s \tau}) + \hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}))$$

Taking the exponential of both sides, then dividing by  $\exp(-0.5\hat{g}_{undef}(1 - e^{-2\theta_s F_s \tau}))$  and multiplying by  $1 - f_{undef}$  leads to:

$$\exp(-0.5(\hat{g}_{undef}(1 - e^{-2\theta_s F_s}) - \hat{g}_{undef}(1 - e^{-2\theta_s F_s \tau}))) - f_{undef} \exp(-0.5\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m})) < (1 - f_{undef}) \exp(-0.5(\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}))) \quad (x)$$

Adding  $f_{undef} \exp(-0.5\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}))$  to both sides and taking the natural logarithms and multiplying by -2 leaves:

$$\hat{g}_{undef}(e^{-2\theta_s F_s \tau} - e^{-2\theta_s F_s}) > \hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m}) \quad (xi)$$

Dividing both sides by  $(e^{-2\theta_s F_s \tau} - e^{-2\theta_s F_s})$  we then obtain:

$$\hat{g}_{undef} > \frac{\hat{m}_{undef}(e^{-2\theta_m F_m} - e^{-2(Q+\tau(1-Q))\theta_m F_m})}{e^{-2\theta_s F_s \tau} - e^{-2\theta_s F_s}} \quad (xii)$$

The numerator of the RHS of equation (xii) is always less than 0 for all  $0 < Q \leq 1$ , and the denominator always positive for  $\tau < 1$ , therefore the statement is true. Hence there exists an  $\hat{m}_{def} - \hat{m}_{undef}$  for all positive  $\hat{g}_{undef}$  such that induction is an ESS.



## APPENDIX B

### THE EFFECTS OF REARING DENSITY ON PUPAL WEIGHT IN THE DIAMONDBACK MOTH.

#### *INTRODUCTION*

Diamondback moths often preferentially lay their eggs on previously damaged *Brassica* plants (Uematsu & Sakanoshita 1993, Chapter 3). Interestingly, this may result in a build-up of larvae on plants already containing larvae. Increased interactions between conspecific larvae in local patches of high larval density may be important for insect population regulation if these interactions have significant density-dependent effects on insect fitness (Fritz 1990).

Harcourt (1986) identified reduced fecundity, pre-pupal parasitism; and small larvae death due to rainfall as the key population regulators of diamondback moth in southern Ontario. Fecundity, the most significant of these factors, was found to be positively correlated with the crude protein content of the cabbage hosts, but Harcourt's analysis of life-table data exposed no evidence of density-dependent effects. There were two weaknesses with the approach that Harcourt took. The first is that in looking for density-dependent effects, the analysis looked for a non-linear relationship between those entering into a class (such as from pupae to adult) and those leaving the class (such as the number of eggs laid). Such an analysis is simplistic in that it does not detect lagged density-dependent effects between previous life stages and the current stage, and so cannot perceive an effect of larval density on fecundity. The second limitation is that larval density and the crude protein content of the leaves may be correlated. In many plants, the % nitrogen content (a correlate of crude protein) of the leaves often decreases during the season as the leaves age, including in cabbage (Scriber & Slansky 1981). At the same time, insect densities of multi-voltine species like diamondback moth will often increase during the season. Hence an effect of larval density on fecundity would be detected as a correlation with crude protein. Given the uncertainty surrounding Harcourt's results, this experiment sought to address whether fecundity in diamondback moth can be dependent on larval density.

## METHODS

### The relationship between pupal weight and fecundity

One way of estimating female fecundity is to measure the pupal mass of females (Schoener 1971). In diamondback moth, pupal mass has been positively correlated with the number of mature eggs in the ovaries of newly emerged females (Hillyer & Thorsteinson 1969). To confirm that pupal mass is a suitable measure of lifetime fecundity, a female and a male diamondback moth were confined on an 18 day old cabbage plant for the duration of the female's life. Females came from one of two weight classes - heavy (8.4 - 9.5 mg) or light (4.8 - 6.5 mg). Of the twenty females in total that were used, four failed to hatch or lay any eggs. It was not possible to distinguish failure to lay due to weight related factors from non-weight related causes such as male infertility. Therefore those moths that did not lay any eggs were excluded from the analysis. Eggs were counted and the plants replaced every five days. Significantly more eggs were laid by heavier females than by lighter females (mean  $\pm$  SE:  $369.0 \pm 33.7$  and  $243.1 \pm 16.4$  respectively; ANOVA, log transformed,  $F_{1,14} = 13.0$ ,  $p = 0.0029$ ). Hence, the use of pupal mass as a measure of relative fecundity in diamondback moth appears to be justified.

### Density effects on pupal weights

To measure the effect of insect density on pupal weight, 40 cabbage plants (*Brassica oleracea* L. cv. "golden acre merit") were grown in fertilised potting mix in the University of Canterbury glasshouses. When the cabbage plants were 21 days old, eggs were transferred to the plants from 8 day old seedlings upon which fifty mated female diamondback moths had been allowed to lay for 24 hours. The eggs were transferred using a camel-hair brush dipped in hypochlorite solution, which both served as a sterilising agent, and helped to keep the eggs on the brush. Care was taken to select eggs from more than one clutch to ensure there wasn't a maternal effect associated with particular replicates. Eggs were transferred at densities of 2, 5, 10 and 20 eggs per plant. Each density was replicated ten times. Following egg transferral, each plant was surrounded by a nylon mesh bag held away from the plant by a wire frame. The bags remained on the plants for the duration of the experiment and served to prevent larval escape. Eggs were censused daily during the first 6 days of the experiment to determine which ones had successfully hatched. Eggs were already three days old by the transfer date, and generally take less than six days in total to hatch inside the glasshouse. Eggs that had not hatched by the end of this period were assumed to be inviable and were removed from the

cages. The census of egg hatch rates gave the baseline larval density. The sizes of individual plants varied and so larval densities were standardised by recording the basal diameter just above the cotyledons for each plant. These basal diameter measurements were calibrated against measures of dry biomass. These biomass measures were obtained by growing plants under similar conditions, harvesting all leaf blades on each plant and drying them in an oven at 55 °C for two days. The dry leaves were weighed to obtain a measure of the total dry leaf biomass and this regressed against stem diameter ( $r^2 = 0.9675$ ,  $F_{1,39} = 1160$ ,  $p < 0.0001$ ; leaf dryweight =  $0.0079e^{0.8045\text{stem diameter}}$ ).

The larvae were allowed to feed until pupation. The mean fresh masses of both male and female pupae were obtained for each plant. The pupae were weighed on the second day following their pre-pupal moult. After all surviving larvae had pupated, the leaf blades of each plant were harvested, dried and weighed to determine the remaining dry biomass.

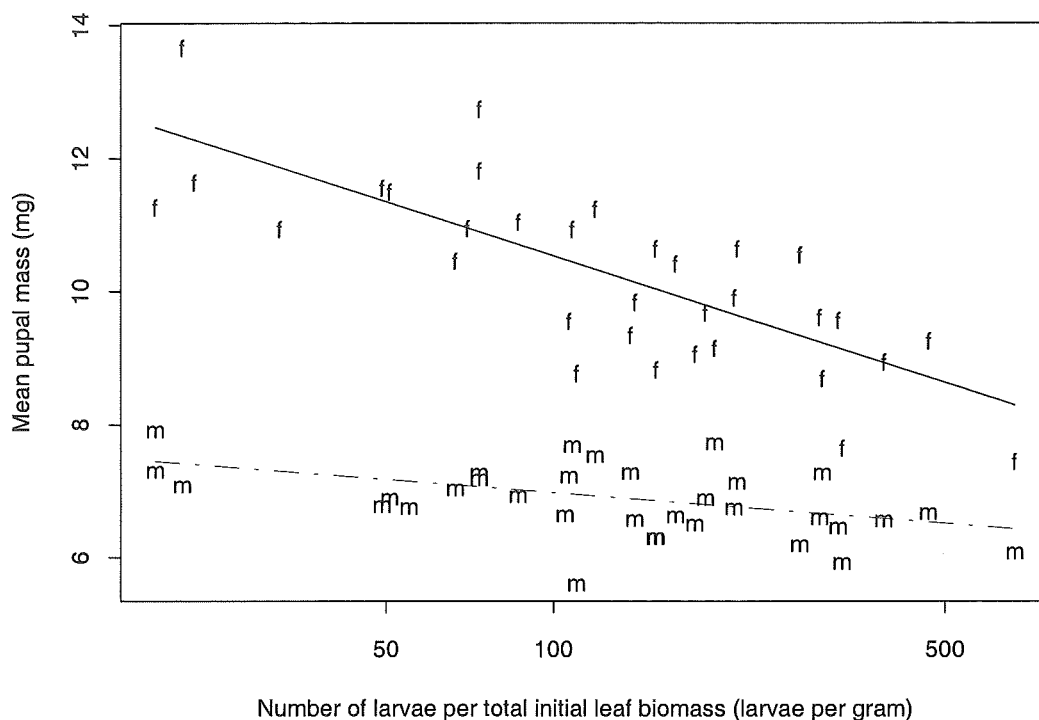
## Analysis

Initial larval density was calculated as the number of eggs that hatched divided by the estimated dry biomass of leaves per plant as computed from the stem diameter measurements. The mean pupal mass of males and females were regressed separately against initial larval density. The proportion of pupae that were female was also included in the initial regression models on the basis that if larger females eat more than males, this may increase any density-dependent effects. The slopes of the relationship between the mean pupal mass and initial larval density for males and females were compared using the method of Zar (1984, p292).

## RESULTS

Larval mortality rates were generally quite low (mean = 6.3%), probably reflecting the glasshouse rearing environment and the absence of natural enemies. No effect of larval density on larval death rate was detected (regression, sqrt transformed,  $r^2 = 0.0861$ ,  $F_{1,33} = 3.109$ ,  $p = 0.087$ ) and so changes in actual densities due to larval death ought not to be a component in any density-dependent relationships with pupal mass.

Larvae reared at low density had significantly higher pupal mass than those reared at high densities (Figure B.1, Table B.1). This observation was consistent both for males and for females. The proportion of pupae that were female was a significant predictor of female, but not male pupal mass. This may illustrate a higher sensitivity to food limitation on the part of



**Figure B.1.** *The mean pupal mass as a function of larval density. The values for females have been corrected for the effect of the proportion of pupae that were female. Legend: f = females; m = males; solid line = female's regression line; alternate dash = male's regression line.*

females, particularly if females require more food than males in order to obtain their higher pupal mass. Increased sensitivity of females is further evidenced by the significantly greater slope with respect to larval density for females relative to males (2-sided  $t$ -test,  $t_{57} = 25.2$ ,  $p < 0.0001$ ).

Most of the consumption of leaves appears to occur during the last two larval instars in diamondback moth (pers. obs.). Therefore another and potentially more relevant way of measuring density stress is to look at the number of pupae relative to the remaining dry biomass after all larvae have pupated. The remaining dry biomass defines the final size of the resource pool, including any effects of interactions between larval density and the plant's growth response such as compensation. Fitting similar models as used for larval density, a significant effect of remaining biomass per pupae on pupal mass was detected for males and females (Table B.1). Using pupal density, instead of larval density, produced a modest increase in the total variation explained by the models.

**Table B.1.** *The regression results for mean pupal mass against density and the proportion of pupae that were female. Note that for the male regression models, the significance of the proportion of female pupae term was evaluated with the density term already fitted, whereas for the density term the proportion term has been omitted and is the same as the final model.*

Terms	F	df	p-value	r <sup>2</sup>	Coefficients (final model only)			
					ln(density)	Prop. female pupae	Constant	
<b>Initial larval density (hatched larvae per g dry matter at start)</b>								
Males (Prop.fem. pupae, model includes ln(density))	0.68	1,30	0.415					
Males (ln(density) alone)	9.03	1,31	0.0052	0.226	-0.2858	0		8.2942
Females (ln(density))	21.7	1,30	<0.0001					
Females (Prop.fem. pupae)	9.44	1,30	0.0045					
Females (ln(density) + Prop.fem. pupae)	15.56	2,30	<0.0001	0.509	-1.1771	-2.2972		15.9488
<b>Final pupal density (pupae per g dry matter at end)</b>								
Males (Prop.fem. pupae, model includes ln(density))	0.87	1,30	0.36					
Males (ln(density) alone)	13.64	1,31	0.0008	0.306	-0.2284	0		7.4143
Females (ln(density))	48.0	1,30	<0.0001					
Females (Prop.fem. pupae)	7.90	1,30	0.0086					
Females (ln(density) + Prop.fem. pupae)	27.98	2,30	<0.0001	0.651	-0.8631	-1.5676		11.8471

## CONCLUSION

The results of this experiment clearly demonstrate that pupal mass and hence fecundity can respond to density-dependent stress. Field studies and perhaps a re-analysis of Harcourt's data (Harcourt 1986) are required to test whether this is the case in natural populations.

## APPENDIX C

## KEY TO THE IMPORTANT SYMBOLS USED IN CHAPTER 4.

Note that the subscript  $x$  refers to a plant phenotype and is either undefended (*undef*) or defended (*def*).

$\alpha$	Severity of induced resistance on a susceptible herbivore's fecundity
$\beta$	Probability that an insect does not accept hosts during the discrimination period
$\delta$	Insect fecundity scalar
$\Phi$	Probability that an insect accepts a host that it is responsive to given that the host has been encountered
$\lambda$	Probability than an induced plant reverts back to the undefended phenotype
$\theta$	Scaling parameter for the cost of herbivore feeding
$\tau$	Induced defence efficiency parameter
$\psi$	Insect search rate
$b$	Stability indicator
$c_u$	Insect density dependent viability parameter for insect of genotype $u$
$D$	Plant death rate
$E_x$	Probability that a plant of phenotype $x$ is accepted for oviposition
$F_{max}$	Maximum fecundity of a susceptible insect
$F_u$	Average clutch size per insect of genotype $u$
$f_x$	Frequency of the undefended phenotype
$\hat{g}_x$	Equilibrium density of non-adapted herbivores on phenotype $x$
$G$	Global mean density of non-adapted herbivores
$g_{critical}$	Critical non-adapted herbivore density at which induction is always stable against undefended invaders
$H_i$	Number of herbivores distributed between $h$ cells
$I_x$	Stimulus intensity of phenotype $x$
$k$	Relative competitive advantage of strategy $A$ over $A'$
$\hat{m}_x$	Equilibrium density of adapted herbivores on phenotype $x$
$M$	Global mean density of adapted herbivores
$P_i$	Proportion of plants adopting strategy $A$ in a population containing $1-P_i$ $A'$ individuals
$Q$	Proportion of the maximum fecundity that the fecundity of partially adapted herbivores is reduced to when reared entirely on defended plants
$r_x$	Realised reproductive output of plant with phenotype $x$
$s_x$	Rate of host specific stimulation to phenotype $x$
$W(A', A)$	Fitness of a single $A'$ strategist in a population of $A$ strategists
$W(A'_\epsilon, A_\epsilon)$	Fitness of an $A'$ strategist in a population of $\epsilon A'$ and $(1-\epsilon) A$ strategists